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Sampling and Analysis of Dynamic Headspace Volatile Compounds From Selected Protein-Rich Food Matrices.

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Matiella, José Ernesto, Ph.D.

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SAMPLING AND ANALYSIS OF DYNAMIC HEADSPACE VOLATILE COMPOUNDS FROM SELECTED PROTEIN-RICH FOOD MATRICES

A Dissertation

Submitted to the Graduate Faculty of the
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in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
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LIST OF ABBREVIATIONS

cm	centimeter
CV	coefficient of variation (standard deviation x 100/mean)
^{o}C	degree Celsius
d	deuterium
DHS	dynamic headspace sampling or system
FPD	Flame photometric detector
g	gram(s)
GC	gas chromatography or gas chromatograph
GC/MS	combined GC and MS
hr	hour(s)
i.d.	inside diameter
I.S.	internal standard
m	meter
min	minute
mL	milliliter
mm	millimeter
MS	mass spectrometry or mass spectrometer
MSD	mass selective detector
ng	nanogram
o.d.	outside diameter
ppb	parts per billion
ppm	parts per million
psi	pounds per square inch
RI	retention index or retention indices

sec	second(s)
USDA	United States Department of Agriculture
μL	microliter(s)

ABSTRACT

Volatile compounds from two protein-rich foods, crabmeat and eggs, were studied with emphasis on qualitative and quantitative determination of a wide variety of chemical substances including degradation compounds from amino acids, lipids and carbohydrates as well as compounds derived from the packaging material and the environment. Boiled and pasteurized blue crab (Callinectes sapidus) meat samples were analyzed for volatile flavor components by dynamic headspace sampling, capillary column gas chromatography and mass spectrometry. Fifty-three volatile compounds were identified in crabmeat samples. Levels of volatile compounds in both samples were compared and it was found that the boiled crabmeat contained higher levels of most compounds.

Thirty-eight volatile compounds were identified in scrambled eggs. Selected compounds were quantified. Aldehydes were the most abundant volatile compounds in the egg samples. Volatile styrene monomer increased in scrambled egg samples during a two-week storage of shell eggs in a polystyrene container. Scrambled eggs prepared from a batch of supermarket shell eggs contained 7 times more ethylbenzene and styrene than those prepared from a batch of fresh farm eggs stored

in a polystyrene container for two weeks.

A USDA method of dynamic headspace sampling/gas chromatography was evaluated and used to quantify dimethyl sulfides as potential quality markers in liquid whole egg samples. Percent acceptance (0, 20, 40, 60, 80, or 100%) by a panel of five USDA egg inspectors on an experimental batch of samples generally decreased with increasing dimethyl sulfide (DMS) concentration (0 to 540 ng/g) in the samples. In addition, dynamics of DMS and dimethyl disulfide (DMDS) formation during egg spoilage were investigated. It was found that egg decomposition may follow different paths and that DMS concentration may not be indicative of the extent of spoilage in a sample.

This study used a relatively low-artifact analytical approach to generate objective information on flavor quality of selected protein-rich foods. Further investigation into the interaction of the critical volatile compounds with the food matrices as well as packaging and/or the environment may provide critical information to facilitate improvement of the flavor quality of the foods.

CHAPTER I

INTRODUCTION

The analysis of critical volatile compounds has gained importance in the food industry since aroma has been recognized as the most important part of a food's perceived flavor (Flath et al., 1981; Jennings, 1981; Cronin, 1982, Heath and Reineccius, 1986). Great progress has been made in this field since GC and MS have been applied to the separation and identification of flavor compound mixtures. Yet, the isolation of aroma compounds from the food matrix has been considered the most difficult and critical step in this type of analysis (Flath et al., 1981; Heath and Reineccius, 1986).

Classical analytical methods for volatile compound isolation include solvent extraction, steam and vacuum distillation (usually followed by extraction), and combinations of the above, such as simultaneous distillation and extraction (Sugisawa, 1981). These techniques require relatively large amounts of sample and tend to be laborious and are prone to the introduction of impurities from the solvent, glassware, antifoaming agent, or distilled water (Lee et al.,

1975; Jeon et al., 1976; Núñez and González, 1984). Besides, these procedures often involve excessive heating of the sample which leads to formation of artifacts (Chang et al., 1977). However, one advantage of these methods is that they yield an amount of extract from each sample that allows characterization and/or quantification by different means.

Headspace sampling techniques include static headspace sampling (SHS) and dynamic headspace sampling (DHS) methods. SHS is a relatively simple procedure: the food is enclosed in an air-tight vessel often for a period of time long enough to allow the migration of volatile compounds from the food into the headspace to reach equilibrium. A sample of the headspace above the food is drawn (through a septum) with a gas-tight syringe and directly injected into the inlet of a GC. This technique has the advantages of minimal sample preparation and low risk of artifact formation (Issenberg, 1969, Dickens, 1979). However, since the concentrations of volatile compounds in static headspace are usually low (Chang et al., 1977), a large injection volume (5 to 10 mL) is usually necessary (Wampler et al., 1985). Injection of relatively large volumes of air and moisture can cause rapid deterioration of chromatographic columns (Jennings and

Filsoof, 1977). In addition, SHS lacks reproducibility (Waltking and Goetz, 1983).

DHS is a more sophisticated technique. Volatile compounds are swept from the headspace over the enclosed food sample with a continuous stream of gas (purging), such as helium or nitrogen, and concentrated by trapping them with a porous sorbent, usually enclosed in a cartridge. Sorbent materials that have been utilized for this purpose include activated charcoal and a variety of porous polymers such as Porapak, Chromosorb, and Tenax (Sydor and Pietryzk, 1978; Boyko et al., 1978). Good sorbents for trapping volatile compounds should have the following physical properties: (1) efficient concentration of volatiles from large headspace samples with minimum water retention; (2) high absorptive capacity; (3) non-specific absorption of volatiles at room temperature; (4) reproducible release of the trapped volatiles during thermal or solvent desorption and non-destructive transfer to chromatographic equipment; and (5) not chemically reactive or catalytic at operating temperatures (Withycombe et al., 1978). Currently, the most commonly used sorbent is Tenax-TA (poly-2,6-diphenyl-p-phenylene oxide, Chrompack, Raritan, NJ). If high-moisture samples are purged, the water that accumulates in the sorbent trap must be removed, as it

interferes with gas chromatograhic and mass spectrometric analysis. Williams et al. (1988) developed an off-line dry-purge procedure for water removal from sorbent traps. This procedure has greatly facilitated the analysis of high-moisture foods. The volatiles trapped in the sorbent cartridge are desorbed either by flash-heating the sorbent cartridge or by solvent elution from the sorbent. After desorption from the sorbent trap, the volatile compounds are transferred to the GC column via a transfer line which is heated to prevent condensation of the compounds in the line. A cryogenic trap consisting of a dry ice/acetone, dry ice/ethanol, or liquid nitrogen bath is used to focus the volatile compounds at the beginning of the column to improve chromatographic performance (Westendorf, 1988).

DHS has become a popular sample isolation technique for the analysis of volatile compounds because it does not require lengthy sample preparation procedures or large amounts of sample. It is also a versatile and low-artifact isolation technique (Vejaphan et al., 1988; Hsieh et al., 1989) that can be applied to a variety of sample matrices, including solids and liquids (Venema, 1988) such as polymers (Moncur et al., 1981), environmental water and soil samples (Bellar et al., 1979), and oils.

The matrix from which the volatile compounds are sampled affects the efficiency and reproducibility of DHS (Venema, 1988). Food structures are generally heterogeneous and food components (water, carbohydrates, proteins, and lipids) tend to bind volatile compounds (Maier, 1975; Kirk, 1988). However, DHS has been successfully utilized in the analysis of aroma compounds in many foods, including vegetable oils (Selke and Frankel, 1987), fish oils (Hsieh et al., 1989b) cereals (Kirk, 1988), seafoods (Hsieh et al., 1988; Vejaphan et al., 1988; Tanchotikul and Hsieh, 1989), fruits (Takeoka et al.,1988; Hsieh et al., 1989a) and nuts (Chang and Hsieh, 1988; Vercellotti et al., 1989), among others.

Recently, a new "aroma trap" technique (Hsieh and Alford, 1990) has been used to trap the equilibrium headspace volatile compounds by placing a small quantity of the Tenax-TA polymer inside a packaged food system. Subsequent desorption of the volatile compounds from the Tenax-TA sorbent afforded convenient analysis to assess the flavor quality of potato chips and breakfast cereals.

Chapters II, III, and IV were written in manuscript format to meet the requirements for publication set by the Journal of Food Science. These three chapters describe applications of DHS to analyses of volatile

components of high-protein/high moisture foods that have undergone different types of processing and/or storage. Chapter II (in press, Journal of Food Science) compares profiles of volatile compounds from boiled and from pasteurized crabmeat samples. Chapter III (submitted for publication, Journal of Food Science) compares the volatile components in scrambled eggs prepared from supermarket shell eggs (packaged in polystyrene), fresh farm eggs, and eggs that have been stored in polystyrene egg cartons for two weeks, in refrigeration. Chapter IV reports an evaluation of a USDA-developed DHS/GC method for the analysis of volatile sulfur-containing compounds as markers of quality in liquid whole eggs.

Chapter V discusses the overall significance of this work and presents concluding remarks.

CHAPTER II

ANALYSIS OF CRABMEAT VOLATILE COMPOUNDS*

José E. Matiella and Thomas C.-Y. Hsieh

ABSTRACT

Boiled and pasteurized blue crab (Callinectes sapidus) meat samples were analyzed for volatile flavor components by dynamic headspace sampling, capillary column gas chromatography and mass spectrometry. Fifty-three volatile compounds were identified including aldehydes, ketones, alcohols, aromatics, furans, sulfur-containing compounds, terpenes, alkanes and miscellaneous compounds. Levels of volatile compounds in both samples were compared and it was found that the boiled crabmeat contained higher levels of most compounds.

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*In press, Journal of Food Science.

INTRODUCTION

The meat from cooked blue crabs (Callinectes sapidus), with its distinctive aroma and taste, has long been appreciated by consumers. The blue crab market was originally limited to coastal regions due to its highly perishable character. The development of pasteurization processes for crab meat extended its shelf-life under refrigeration from 7-10 days to approximately 12 months. Pasteurization alleviated the inventory and distribution problems that limited the expansion of the crab meat market. However, problems of loss of desirable flavor, formation of off-flavor compounds and discolorations ("bluing") during storage still occur (Burnette and Flick, 1978; Wicker and Acton, 1981; Ward et al., 1982).

The major non-volatile taste-active compounds in boiled snow crab meat have been identified as glycine, alanine, arginine, glutamic acid, inosine 5'-mono-phosphate (IMP), adenosine 5'-monophosphate (AMP), guanosine 5'-mono-phosphate (GMP), NaCl and K_2HPO_4 (Konosu and Fuke, 1982; Hayashi et al., 1984). However, little information on the volatile flavor compounds of blue crab meat can be found in the literature. The formation of off-flavor compounds in blue crab meat during storage has been a problem for many years (Burnette and Flick, 1978). The most common

decomposition off-flavor compound in seafoods may be ammonia, which gives a very disagreeable odor. Kubota et al. (1980a) identified certain carbonyl compounds, pyrazines and some sulfur-containing compounds responsible for the rancid-nutty odor of cooked Antarctic krill. Other sulfur-containing compounds in krill produced garlic- or onion-like odors (Kubota et al., 1980b). Freeman et al. (1985) successfully used gamma irradiation to remove the garlic-like odor of bis-(methylthio)-methane from lobsters and prawns.

Vejaphan et al. (1988) used dynamic headspace sampling/gas chromatography/mass spectrometry (DHS/GC/MS) to analyze volatile flavor compounds in boiled crayfish (Procambarus clarkii) tail meat. Tanchotikul and Hsieh (1989) used DHS/GC/MS to identify volatile compounds in crayfish waste from a processing plant. The analysis of volatile compounds in the dynamic headspace of foods offers the advantages of reduced sample size and simple sample preparation, as well as low risk of artifact formation as compared with steam distillation, solvent extraction and other methods (Vejaphan et al., 1988).

The objectives of this study were: (1) to identify and (2) to compare the profiles of volatile compounds of boiled and of pasteurized blue crab meat by DHS/GC/MS.

MATERIALS AND METHODS

Materials

Live blue crabs were purchased from a local seafood retailer. Canned, pasteurized and refrigerated blue crab meat was obtained from Klein's Seafood, Inc. (Baton Rouge, LA). Most standard flavor compounds were purchased from commercial sources. Some standards were generous gifts from the Flavor and Fragrances Division of Aldrich Chemical Co., Inc. (Milwaukee, WI).

Sample preparation

Boiled crabmeat. Live blue crabs were boiled for 10 min. The white crabmeat was picked and stored in a stainless steel bowl in an ice bath to prevent spoilage during picking. The meat was divided into 50g-portions and each portion was spiked with 333 μL of a 5 ng/μL solution of internal standard (I.S.) ethylbenzene-d_{10} (Cambridge Isotope Lab., Woburn, MA) to achieve a concentration of 1000ng of I.S. per 30g of meat. Each 50-g portion of spiked meat was then homogenized using a porcelain mortar and pestle in an ice bath. All of the homogenized meat was then mixed together and homogenized again to assure sample uniformity. The entire sample was then divided into 3.5g aliquots, placed into vials (17 mm o.d. x 60 mm height) fitted

with teflon cap liners, frozen and placed in an ultra-freezer at -85°C.

Pasteurized crabmeat. Cans of pasteurized crabmeat (227g each), stored at 4°C for 55 days from the date of pasteurization, were opened and the meat was portioned, spiked with the internal standard, homogenized, mixed, divided into aliquots and frozen as described for the boiled crabmeat.

Dynamic headspace sampling (DHS)

A 3.0g sample of crabmeat homogenate was obtained from each 3.5g aliquot (after thawing) and placed into a headspace sampling tube (15.2 cm length x 1.6 cm i.d.). The latter was attached to a DHS system as shown in Figure 1. Ultra high purity helium (99.999%, Linde Div., Union Carbide Corp., Danbury, CT) served as sample purge and trap desorption gas in the DHS system, and as the carrier gas for gas chromatography (GC). An oxygen scrubber (Tekmar Co., Cincinnati, OH) and a hydrocarbon trap were installed in the helium line. The sample was preheated for 2 min, without gas flow, in a water bath at 50°C. The temperature of the sample fitting in the DHS system (Figure 1) was maintained at 125°C by a heating tape (Thermolyne Corp., Dubuque, IA). The volatile compounds purged from the sample were trapped onto Tenax TA™ (0.24g, 60-80 mesh,

Chrompack, Raritan, NJ) sorbent packed in stainless steel tubing (0.3 cm o.d. x 30.5 cm). Each of the 10 sample tubes was purged for 30 min at a temperature of 50°C, with a purge gas flow of 40 mL/min. After purging, the trap loaded with the volatile compounds was removed from the DHS system and dry-purged using an off-line procedure (Williams et al., 1988) to remove the moisture accumulated during sample purging. Off-line dry-purge (60 mL/min) was continued until the trap returned to its initial weight before sample purging. A Tekmar 4000 headspace concentrator was used for desorption of the trapped compounds.

Gas chromatography/mass spectrometry (GC/MS)

Desorption of the trapped volatile compounds was accomplished by flash-heating the Tenax trap to 185°C. The GC vents for septum purge and split flow were closed and the carrier gas pressure increased from 12 psi to 35 psi to facilitate the transfer of desorbed volatile compounds to the gas chromatograph through a transfer line heated at 120°C. During a 15-min desorption period, the volatile compounds were cryogenically focused at the beginning of the column by keeping a 15-cm section of the column in a liquid nitrogen bath. A fused silica capillary column (Supelcowax 10, 60 m length x 0.25 mm i.d. x 0.25 μm

film thickness, Supelco, Inc., Bellefonte, PA) was used. Other GC/MS conditions have been described elsewhere (Vejaphan et al., 1988). All analyses were run in triplicate.

Compound identifications

Identification of the volatile compounds by linear GC retention indices (van den Dool and Kratz, 1963) and MS was performed as described by Vejaphan et al. (1988).

Relative abundance and peak areas of coeluting compounds

The relative abundance of each compound identified was estimated by the ratio of its total ion peak area to that of the I.S. ethylbenzene-d_{10}. The total ion peak areas of coeluting compounds were estimated by mass chromatography (Hites and Biemann, 1970) and the equation described by Tanchotikul and Hsieh (1989).

Quantification of compounds

Selected positively identified compounds were quantified by determination of calibration curves of peak area ratios (analyte/I.S.) vs. amount ratios (analyte/I.S.) of standard solutions analyzed under

comparable GC/MS conditions. The concentration of an analyte in the sample was calculated as follows:

$$C = \frac{A \times B}{W}$$

where C = concentration (ng/g); A = amount ratio of analyte/I.S.; B = amount of I.S. (1000ng); and W = weight of sample (30g).

RESULTS AND DISCUSSION

A DHS/GC/MS method was used to analyze volatile compounds from boiled and pasteurized crabmeat for objective flavor quality assessment. Typical total ion chromatograms are shown in Figures 2 and 3 for boiled and pasteurized crabmeat, respectively. A total of 35 volatile compounds (Table 1) were positively identified. In addition, 18 compounds were tentatively identified based on MS data only. Among the various compounds detected were three alcohols, four aldehydes, three normal alkanes, 30 aromatic compounds, two furans, four ketones, two sulfur-containing compounds, two terpenes, and three miscellaneous compounds.

Four saturated aldehydes (C5 to C7) were identified. Three of these compounds were present in greater amounts in boiled crabmeat than in pasteurized crabmeat (3-methyl-butanal: twice, pentanal: 4 times, and

hexanal: more than 3 times as much). Heptanal was found in the pasteurized meat only. These compounds have been reported previously in fish (Josephson et al., 1983 and 1984), krill (Kubota et al., 1982), crayfish (Vejaphan et al., 1988, Tanchotikul and Hsieh, 1989) and shrimp (Kubota et al., 1986). The 3-methylbutanal was the most abundant of its class, followed by pentanal, in both the boiled and pasteurized crabmeat. These two aldehydes have similar aroma characteristics and have been reported to produce green, fruity, nutty, cheesy, or sweaty odors, depending on the dilution (Fors, 1983; Arctander, 1969).

Four saturated ketones including 2-pentanone, 4-methyl-2-pentanone, 3-hexanone and 2-hexanone were found in both the boiled and pasteurized crabmeats. These compounds were approximately two to 24 times more abundant in the boiled crabmeat. The 2-pentanone has been previously reported in fish (Josephson et al., 1984), krill (Kubota et al., 1982) and shrimp (Kubota et al., 1986). The 2-hexanone also has been found in crayfish (Vejaphan et al., 1988). Kubota et al. (1986) have found a large number of ketones, mostly C_4-C_8 aliphatic ketones, in the volatiles of roasted shrimp and proposed that they may have originated from fat degradation during heating.

Three saturated alcohols were identified in the crabmeat samples. However, 1-butanol was present in the pasteurized crabmeat only. The other two alcohols, 2-hexanol and 3-hexanol, were found in both boiled (2 to 3 times more abundant) and pasteurized crabmeat. According to Heath and Reineccius (1986), the saturated alcohols usually make minor contributions to flavor in food systems, unless they are present in relatively high concentrations.

Toluene, 1,4-dichlorobenzene and other aromatic compounds (Table 1) were found in the samples. The source of these compounds was not clear. However, the possibility of analytical procedural artifacts contributing to these benzene derivative peaks was ruled out, since procedural backgrounds were thoroughly examined under comparable experimental conditions before sample analysis.

Two alkylfurans were identified. 2-Ethylfuran was found in the pasteurized crabmeat only. This furan has been reported to have a warm sweet aroma when diluted (Arctander, 1969; Fors, 1983). The 2-pentylfuran was found in both the boiled and pasteurized crabmeats at a concentration of less than 2ng/g. This compound has been reported as an off-flavor in certain fats and oils, imparting a beany, grassy odor characteristic of

the reversion flavor of soybean oil (Krishnamurthy et al., 1967).

Two sulfur-containing compounds were identified in both boiled and pasteurized crabmeats. Dimethyl disulfide was more than twice as abundant in the boiled crabmeat and has been reported in crayfish (Vejaphan et al., 1988, Tanchotikul and Hsieh, 1989) and shrimp (Kubota et al., 1986). The odor of dimethyl disulfide has been described as onion- or cabbage-like (Fors, 1983). The heterocyclic 2-methylthiophene also has been reported to have an onion or gasoline-like odor (Fors, 1983). This compound was approximately 35 times more abundant in the pasteurized crabmeat. 2-Methylthiophene has been identified in boiled crayfish tail meat by Vejaphan et al. (1988).

Two monoterpenes were found in both the boiled and pasteurized crabmeat. Limonene produces an agreeable fresh, light and sweet citrusy aroma (Arctander, 1969) and has been reported in crayfish (Vejaphan et al., 1988), in fish (Josephson et al., 1984), in krill (Kubota et al., 1982) and in shrimp (Kubota et al., 1986). Limonene was found to be almost 3 times more abundant in the boiled crabmeat than in the pasteurized product. A second terpene, 5 times more abundant in the pasteurized crabmeat, was found at retention index

1283 but could not be positively identified due to lack of reference standard compounds.

Three n-alkanes including nonane, decane, and undecane were identified. Grosch (1982) and Flath et al. (1981) reported that alkanes do not contribute to flavor in foods, which is consistent with the observations of Vejaphan et al. (1988) in boiled crayfish tail meat.

Three miscellaneous volatile compounds were identified. The unsaturated hydrocarbon 2,5-dimethyl-2,4-hexadiene was detected at approximately 7 times greater concentration in the boiled crabmeat. The alkyl halide dibromomethane was tentatively identified in the pasteurized crabmeat only. The 1H-pyrrole, a nitrogen-containing heterocyclic compound, was identified in the pasteurized crabmeat only. Vejaphan et al. (1988) reported 1H-pyrrole in boiled crayfish tail meat.

Lower concentrations or relative abundances of most of the volatile compounds identified were found in the pasteurized than in the boiled crabmeat. Unfortunately, odor detection threshold data of volatile compounds based on the crabmeat matrix are not available in the literature. Further research will be necessary to determine the sensory characteristics of the volatile compounds found in crabmeat.

Table 1. Volatile compounds identified in boiled and pasteurized blue crab meat.

Peak No.	Compound name by class	RI	Boiled Peak Area Ratio	Boiled Conc. ng/g	Pasteurized Peak Area Ratio	Pasteurized Conc. ng/g
	ALDEHYDES					
2	3-methylbutanal[c,k,s]	911	0.88	54	0.47	27
6	pentanal[c,f]	982	0.71	47	0.16	9
14	hexanal[c,f,k]	1086	0.13	4	0.04	<1
24	heptanal[c,f,k]	1194	n.d.		<0.01	
	KETONES					
5	2-pentanone[f,k,s]	977	0.28		0.06	
8	4-methyl-2-pentanone	1009	0.33	15	0.04	<1
11	3-hexanone	1053	0.23	9	0.14	4
13	2-hexanone[c]	1084	0.31		0.09	
	ALCOHOLS					
21	1-butanol*[c]	1163	n.d.		0.01	
27	3-hexanol*	1222	0.03		0.01	
31	2-hexanol*	1243	0.02		<0.01	
	AROMATICS					
3	benzene[c]	938	0.09		0.02	
10	toluene[c]	1042	0.53		1.56	
17	ethylbenzene-d_{10} (I.S.)	1124	1.00		1.00	
18	ethylbenzene[c]	1128	0.39	14	0.08	3
19	p-xylene[c]	1136	0.49		0.08	
20	m-xylene[c,f,k]	1143	0.94		0.17	
23	o-xylene[c,k]	1190	0.67		0.10	
26	propylbenzene[c]	1213	0.05		0.01	
28	4-ethyltoluene	1227	0.23		0.03	
29	3-ethyltoluene	1230	0.47		0.05	
32	1,3,5-trimethylbenzene[c]	1249	0.24		0.02	
33	styrene[c,f]	1265	0.09		0.03	
34	2-ethyltoluene	1266	0.17		0.01	
35	p-cymene[c]	1274	0.07		0.10	
37	1,2,4-trimethylbenzene[c]	1285	0.87		0.07	
38	a C4-alkylbenzene*	1305	0.03		<0.01	
39	a C4-alkylbenzene*	1306	0.05		0.01	
40	a C4-alkylbenzene*	1309	0.02		<0.01	
41	a C4-alkylbenzene*	1316	0.02		<0.01	
42	a C4-alkylbenzene*	1329	0.08		0.01	
43	1,2,3-trimethylbenzene[c]	1343	0.25		0.02	
44	anisole	1354	0.01		<0.01	

Table 1. Continued.

Peak No.	Compound name by class	RI	Boiled Peak Area Ratio	Boiled Conc. ng/g	Pasteurized Peak Area Ratio	Pasteurized Conc. ng/g
	AROMATICS (continued)					
45	a C4-alkylbenzene*	1358	0.06		<0.01	
46	a C4-alkylbenzene*	1365	0.05		<0.01	
47	a C4-alkylbenzene*	1373	0.08		<0.01	
48	a C4-alkylbenzene*	1417	0.02		n.d.	
49	a C4-alkylbenzene*	1431	0.04		<0.01	
50	a C4-alkylbenzene*	1441	0.09		<0.01	
51	1-methyl-2-(2-propenyl)-benzene*	1446	n.d.		0.02	
52	1,4-dichlorobenzene[c]	1453	1.88	57	0.19	6
54	naphthalene[c,f]	1762	0.07		0.01	
	FURANS					
4	2-ethylfuran	952	n.d.		0.02	
30	2-pentylfuran[c]	1237	0.01	<1	0.01	<1
	SULFUR COMPOUNDS					
12	dimethyl disulfide[c,s]	1077	0.26	11	0.11	5
15	2-methylthiophene[c]	1097	0.01		0.35	
	TERPENES					
25	limonene[c,f,k,s]	1198	0.33	10	0.12	4
36	a terpene*	1283	0.01		0.05	
	ALKANES					
1	nonane[c]	900	0.02		0.03	
7	decane[c]	1000	0.13		0.16	
16	Undecane[c,f]	1100	0.14		n.d.	
	MISCELLANEOUS					
9	2,5-dimethyl-2,4-hexadiene	1032	0.21		0.03	
22	dibromomethane*	1174	n.d.		0.04	
53	pyrrole[c]	1530	n.d.		0.02	

RI = retention index.
* = tentatively identified based on MS data only.
n.d. = not detected.
c = reported in crayfish by Vejaphan et al., 1988.
f = reported in fish by Josephson et al., 1984.
k = reported in krill by Kubota et al., 1982.
s = reported in shrimp by Kubota et al., 1986.

Figure 1. Dynamic headspace sampling (purge-and-trap) system utilized in this study (drawing not to scale).

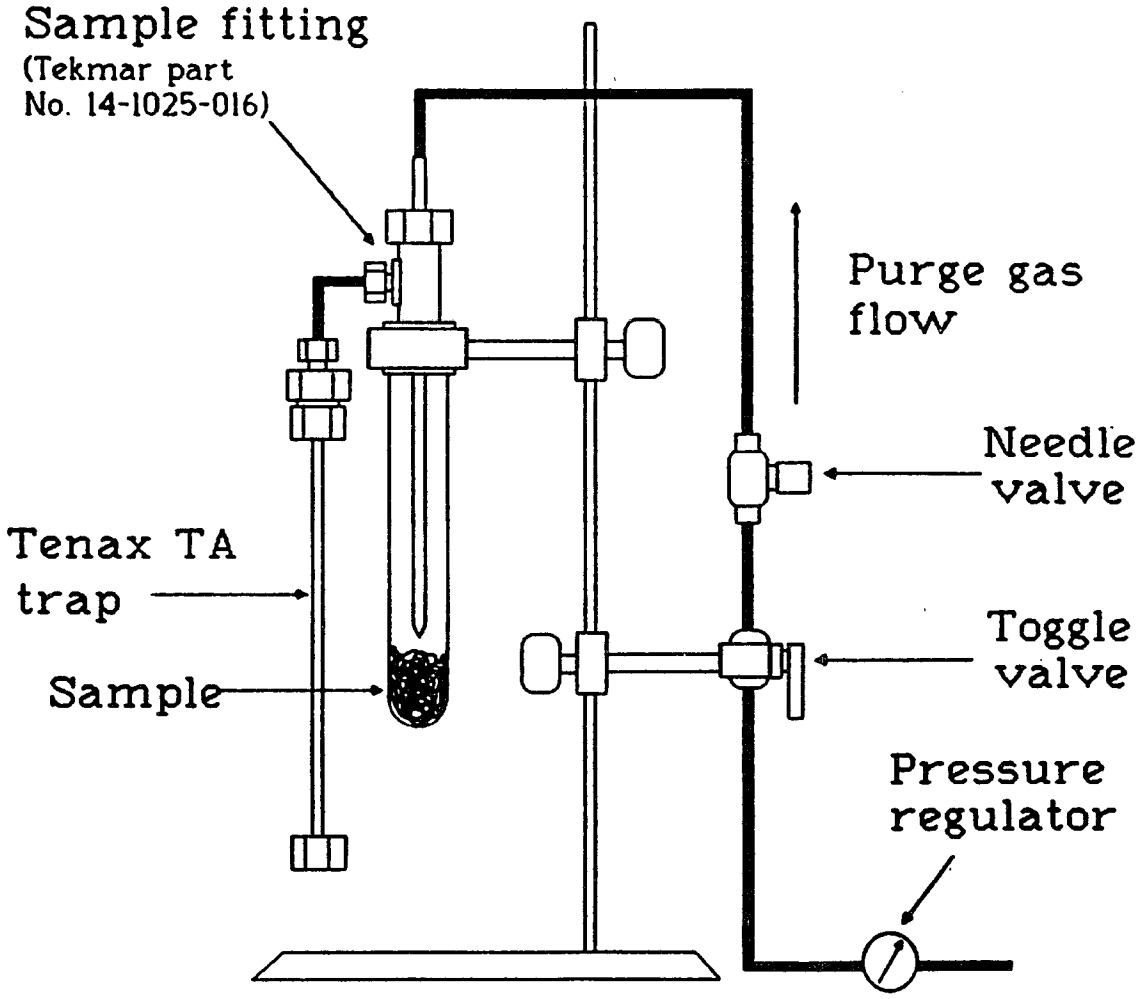

Sample fitting
(Tekmar part
No. 14-1025-016)
Purge gas
flow
Needle
valve
Tenax TA
trap
Toggle
valve
Sample
Pressure
regulator

Figure 2 (A-C). Total ion chromatogram of dynamic headspace volatile components of boiled crabmeat. Peak numbers correspond to those listed in Table 1.

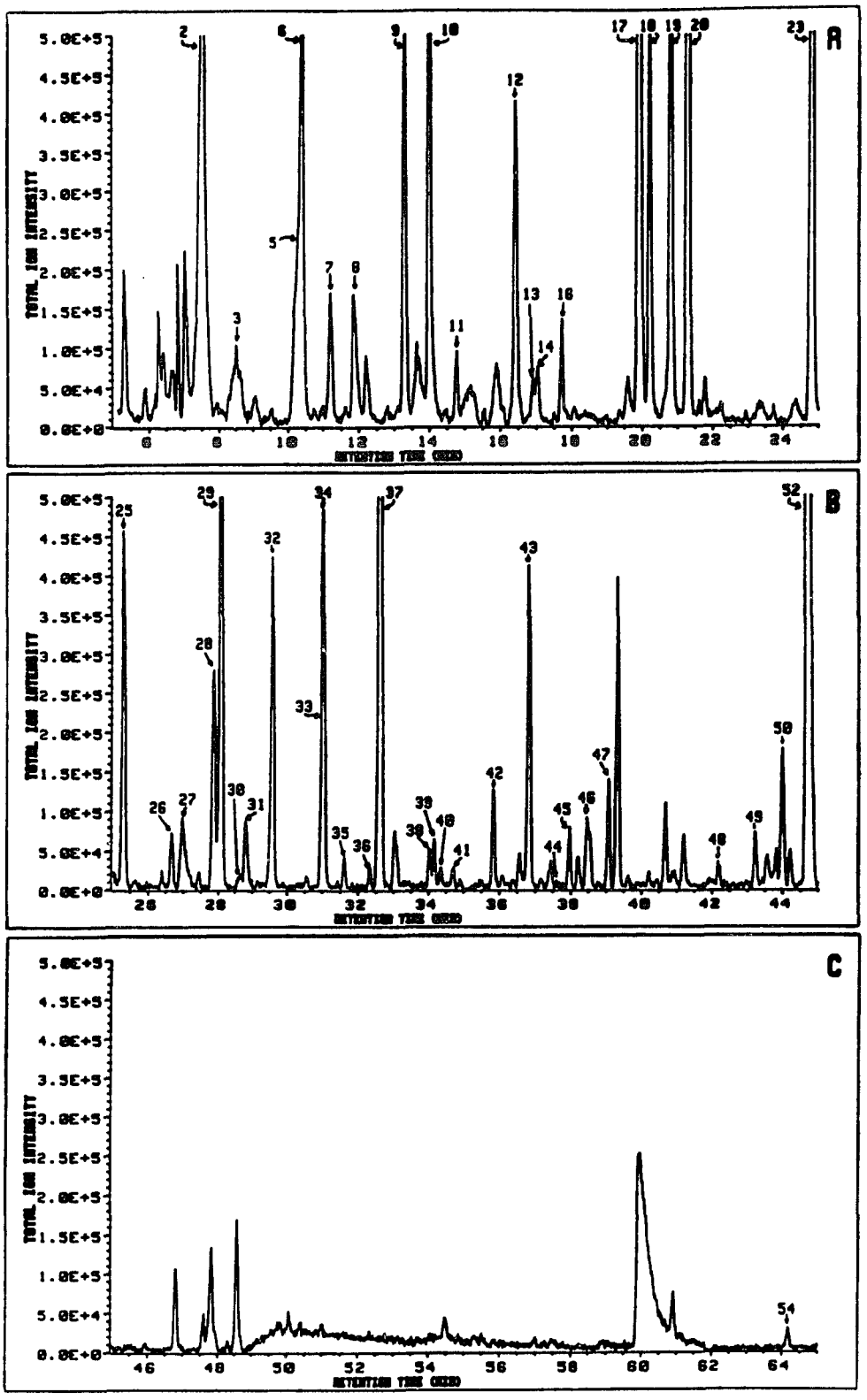
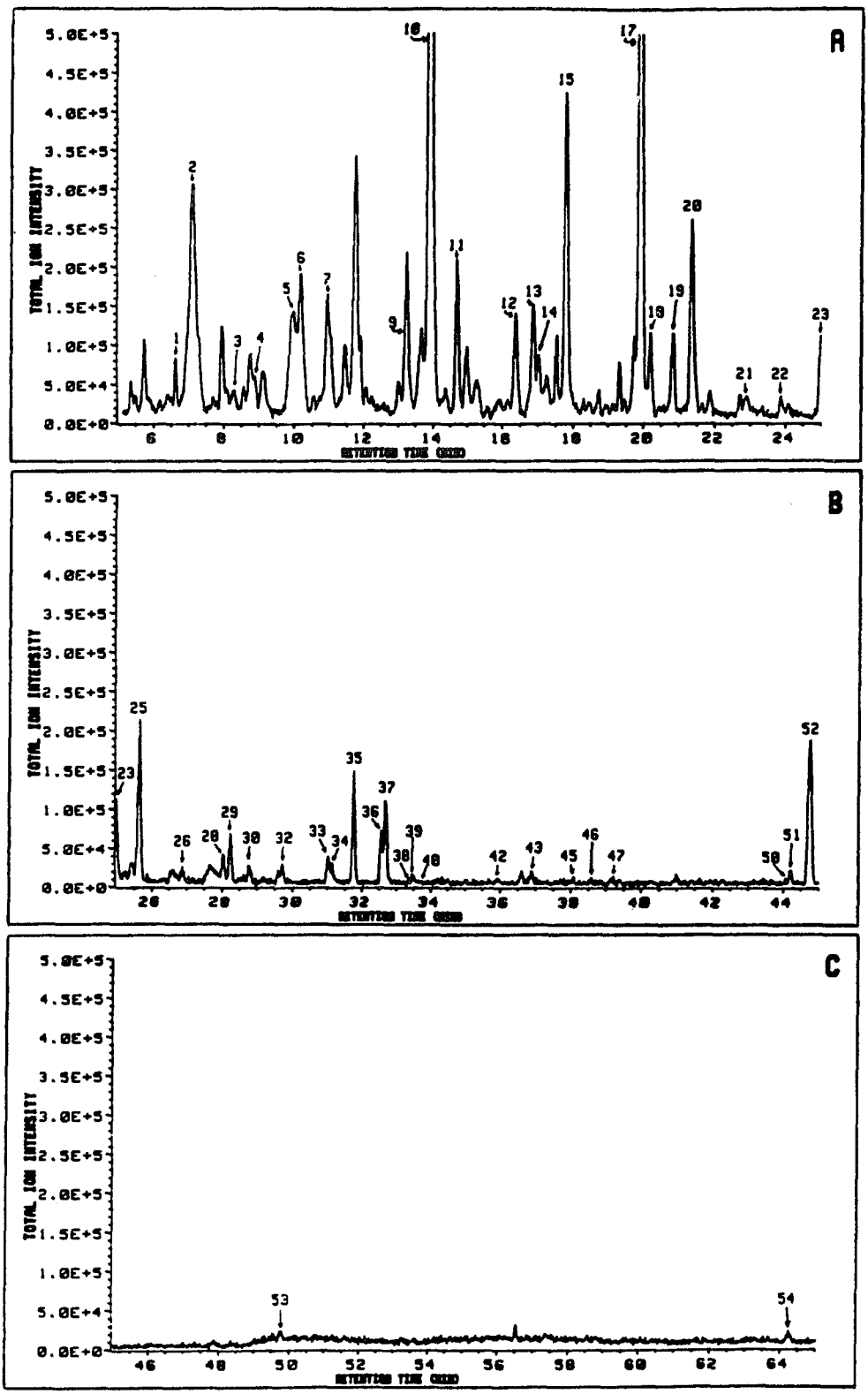

A
B
C
TOTAL ION INTENSITY
RETENTION TIME (MIN)

Figure 3 (A-C). Total ion chromatogram of dynamic headspace volatile components of pasteurized crabmeat. Peak numbers correspond to those listed in Table 1.


A
B
C
TOTAL ION INTENSITY
RETENTION TIME (MIN)

CHAPTER III

ANALYSIS OF VOLATILE COMPOUNDS IN SCRAMBLED EGGS*

José E. Matiella and Thomas C.-Y. Hsieh

ABSTRACT

Volatile compounds in scrambled eggs were analyzed by dynamic headspace sampling, high resolution gas chromatography and mass spectrometry. Thirty eight volatile compounds were identified including aldehydes, ketones, alcohols, furans, esters, benzene derivatives, alkanes, sulfur-containing compounds and a terpene. Selected compounds were quantified. Aldehydes were the most abundant volatile compounds in the egg samples. Volatile styrene monomer increased in scrambled egg samples during a two-week storage of shell eggs in a polystyrene container. Scrambled eggs prepared from a batch of supermarket shell eggs contained 7 times more ethylbenzene and styrene than those prepared from a batch of fresh farm eggs stored in a polystyrene container for two weeks.

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INTRODUCTION

Scrambled chicken eggs have traditionally been an important part of the American diet, mainly as a breakfast dish. Yet, compared with other foods, research on scrambled egg flavor compounds has been neglected (Chen and Hsu, 1981). As egg substitutes and convenience egg products become popular, information on volatile compounds in scrambled eggs may facilitate product development and quality assurance. Flavor quality problems of these products, such as off-flavors and lack/loss of desirable flavors, have been reported (Cotterill, 1986). Egg flavor research published to date has concentrated mostly on volatile compounds from overheated and/or overcooked eggs (Kato et al., 1978; MacLeod and Cave, 1975 and 1976; Umano et al., 1990). The high temperature-long heating time approach generates large numbers of volatile compounds (Parliment et al., 1989). Yet, the cooking time for scrambled eggs in a skillet is usually 2-3 minutes. Umano et al. (1990) stated that some of the volatile compounds identified in their study may not be present in normally cooked eggs. Little information is available on the volatile components of scrambled eggs. Chen and Hsu (1981) analyzed the hydrogen sulfide, methanethiol, ammonia, and total saturated and unsaturated carbonyl compounds in pan scrambled

mixtures of whole egg and albumen by colorimetric methods.

An area of concern when dealing with the flavor quality and safety of foods has been the migration of undesirable volatile compounds from packaging materials into the food. The absorption of certain volatile compounds through the egg shell has been investigated by Kato et al. (1971) and MacLeod and Cave (1977). In recent years, there has been an increase in the use of plastic polymers as food packaging materials. Today, the majority of eggs sold in retail markets are packaged in polystyrene foam containers. Eiceman and Carpen (1982) reported the presence of ethylbenzene, styrene, C3 and C4 alkylbenzenes among other impurities found in polystyrene containers. These volatile compounds may be absorbed through the egg shell.

The objectives of this study were (1) to identify volatile compounds in scrambled egg samples; and (2) to compare the levels of volatile compounds in scrambled eggs prepared from eggs packaged in polystyrene egg cartons and fresh farm eggs never packaged in polystyrene.

MATERIALS AND METHODS

Materials

One dozen eggs, packed in a polystyrene foam container, and corn cooking oil were bought from a local supermarket. Supermarket egg samples (batch S) were prepared immediately after purchase. Freshly laid eggs were obtained from the LSU Poultry Farm and divided into two batches. To assess the migration of styrene and other volatile compounds from the package through the egg shell, one batch of eggs was cooked and prepared for analysis immediately after purchase (batch A). The other batch (batch B) was placed in a commercial polystyrene egg carton and stored at 4^{o}C for two weeks before sample preparation.

Sample preparation

Scrambled Eggs. Each batch of eggs were processed separately. Eggs were shelled and homogenized in a stainless steel bowl, using a hand-held mixer. The mixed eggs were cooked in a Teflon-coated skillet, using corn oil, with constant manual agitation to facilitate even heating. After cooking, the eggs were placed in a stainless steel bowl and kept on ice during the rest of the sample preparation procedure. Fifty-gram scrambled egg portions were homogenized in a porcelain mortar. Samples were spiked with 333 μL of a

solution containing 5 ng/μL of the internal standard (I.S.) ethylbenzene-d_{10}, to obtain a final concentration of 1000ng of I.S. per 30g of egg sample. Each 50-g spiked egg portion was further homogenized using the porcelain mortar and pestle in an ice bath. All of the spiked and homogenized egg portions were then combined and mixed thoroughly in a stainless steel bowl, using the porcelain pestle. The entire egg sample was divided into 3.5g aliquots and transferred into vials (17 mm o.d. x 60 mm height) fitted with Teflon cap liners, and stored in a freezer at -25°C.

Heated oil. Two corn oil batches were heated at the smoking point, in a Teflon coated skillet, for 30 and 60 sec, respectively. Each hot oil batch was rapidly cooled by placing the skillet in an ice bath immediately after heating. Then, 25-g portions of oil were spiked with 50 μL of a 1000 ng/μL solution of I.S. to obtain a concentration of 1000 ng I.S. per 0.5g of oil sample. Each spiked portion was then divided into 1-g aliquots weighed into vials and stored frozen the same way as the scrambled egg samples.

Egg packaging material. Samples of the polystyrene egg containers were cut into small slivers (approx. 5 mm long x 2mm wide). No internal standard was added to these samples.

Dynamic headspace sampling (DHS)

Scrambled eggs. A 3.0g sample of spiked scrambled egg homogenate was taken from a 3.5g aliquot and placed into a headspace sampling tube (15.2 cm length x 1.6 cm i.d.). The latter was attached to a DHS system as described by Matiella and Hsieh (1990). The sample tube was prepurged with helium (99.999%) at 40 mL/min for 2 min without heating. Then it was preheated in a water bath, without purging, for two minutes, to reach the sample purging temperature of 65°C. The volatile compounds were purged for 30 min and concentrated onto a trap consisting of Tenax-TA sorbent packed in stainless steel tubing (0.3 cm o.d. x 30.5 cm). The sample purging procedure was repeated 10 times, for a total sample weight of 30g. An off-line dry-purge procedure was used to remove sample moisture accumulated in the trap, according to Williams et al. (1988). The procedure for desorption of the trapped volatile compounds has been described by Matiella and Hsieh (1990).

Heated oil. A single sample tube with 0.5g of oil was purged in the DHS system, as described above, for each analysis. Dry purging was not necessary because of the low moisture content of the oil.

Egg packaging material. One 0.1-g sample of polystyrene slivers was purged for each analysis. No dry-purging was necessary.

Gas chromatography/mass spectrometry (GC/MS)

GC/MS separation and identification of volatile compounds were performed as described by Vejaphan et al. (1988). Quantification of selected compounds was carried out as described by Matiella and Hsieh (1990).

RESULTS AND DISCUSSION

A typical total ion chromatogram of the dynamic headspace volatile compounds in scrambled eggs is depicted in Figure 4. A total of thirty-eight compounds (Table 2) were identified including six aldehydes, two ketones, four alcohols, two furans, two esters, 15 benzene derivatives (including styrene), four alkanes, two sulfur-containing compounds, and one terpene (limonene). Concentrations and area ratios were corrected so that contributions of trace quantities of certain volatile compounds in the analytical system's background were excluded in Table 2. The volatile compounds detected in both the heated corn oil and the scrambled egg samples are listed in Table 3. Table 4 lists the volatile compounds identified in the polystyrene container material.

Saturated aldehydes (C5 to C9) were the most

abundant compounds identified in the scrambled eggs. The 2-methylbutanal was most abundant in batch S, followed by batch B egg samples. MacLeod and Cave (1975 and 1976) identified 2-methylbutanal in egg SDE extracts as the most abundant volatile component of egg yolk. The 3-methyl-butanal has not been previously reported in eggs. However, the concentrations of 3-methylbutanal detected in the scrambled eggs were 122 ng/g in batch S, 126 ng/g in batch A, and 16 ng/g in batch B. This compound was the most abundant aldehyde in egg samples from batches S and A. A trace of 3-methylbutanal was detected in the polystyrene package, but it was not detected in the heated oil samples. Hence, it most likely originated in the eggs, from which some of it might have migrated from the intact eggs to the package through the shell. The odor of this compound has been described as fruity, fermented, or burnt cheese, with roasted cocoa or coffee notes. Odor detection threshold values reported for 3-methylbutanal are 0.2 to 7 ppb in water and 13 ppb in oil (Fors, 1983). Threshold information for the scrambled egg matrix is not available in the literature. This compound has been previously identified in cooked pork liver (Mussinan and Walradt, 1974), crayfish (Vejaphan et al.,1988; Tanchotikul and Hsieh, 1989), crabmeat (Matiella and Hsieh, 1990) and

other foods of animal origin, and it may be an important component of scrambled egg flavor. Pentanal, hexanal, octanal, and nonanal were present in the scrambled eggs, the heated oil, and the package material samples. These aldehydes could have originated from lipid oxidation of both the egg and the cooking oil during cooking and, to a lesser extent, during storage before cooking. Umano et al. (1990) also identified these four aldehydes in egg samples heated at 200^{o}C.

Two ketones were identified in the egg samples. The 4-methyl-2-pentanone, previously reported in heated eggs (Umano et al., 1990), was detected at very low concentrations in batch B and batch S egg samples. 3-Hexanone was present in all of the egg samples. Its concentration was highest in batch A eggs, followed by batch B and batch S samples. This ketone has not been previously reported in egg samples; but it has been found in industrial crayfish waste (Tanchotikul and Hsieh, 1989) and in crabmeat (Matiella and Hsieh, 1990).

Four saturated alcohols were identified. The 2-propanol, 3-hexanol, and 2-hexanol were in batch A and batch B egg samples. It was not clear why 1-pentanol was found in much higher abundance in the heated oil samples than in batch A and batch B eggs.

However, saturated alcohols usually make minor contributions to flavor in food systems, unless they are present in relatively high concentrations (Heath and Reineccius, 1986).

Two alkylfurans, 2-ethylfuran and 2-pentylfuran, were detected in trace amounts in the egg samples. Both have been previously reported in heated eggs (Umano et al., 1990). Both furans also were present in the corn oil samples. 2-Pentylfuran was found at a concentration of 93 ng/g in the corn oil overheated for 30 sec. It has been reported as imparting the beany, grassy off-flavor characteristic of the reversion of soybean oil (Krishnamurthy et al., 1967). However, the concentration of 2-pentylfuran found in the corn oil was well below the odor threshold in oil (2 ppm) as reported by Fors (1983).

Two esters, methyl butyrate and butyl acetate, were identified in relatively low abundance in the batch B eggs only. These two compounds have been previously reported in fermented egg samples (Bullard et al., 1978). The source of these esters in the scrambled egg samples was not clear. Benzene and 14 other aromatic compounds were found in the scrambled egg samples. A number of these compounds also have been reported in cooked egg samples by MacLeod and Cave (1975 and 1976) and by Umano et al. (1990). Analyses

of material from the polystyrene egg cartons showed that ethylbenzene and styrene were the two major volatile compounds present in these packages (Table 3). All of the aromatic compounds detected in the egg samples also were found in the polystyrene container material. The concentration of styrene in the batch B egg samples was 14 times that in batch A. Apparently, the concentration of styrene increased with time of storage in polystyrene containers. However, ethylbenzene remained the same from batch A to batch B. Batch S samples were found to contain over seven times as much styrene as batch B samples. Similar findings were made in the case of ethylbenzene. MacLeod and Cave (1977) found the time of exposure to be a critical factor affecting the absorption of volatile compounds through the egg shell. In a survey of styrene levels in British foods packaged in polystyrene containers, Gilbert and Startin (1983) reported that raw eggs contained less than 1 ng styrene/g egg. However, the supermarket eggs analyzed in this study contained an average of 103 ng styrene/g egg after cooking. Storage time of batch S eggs and the amount of residual styrene monomer in the polystyrene container were unknown. However, storage was probably long enough to allow the absorption of that much styrene from the package through the egg shell. Most of the aromatic

hydrocarbons found in batch A eggs, which had not been in contact with any polystyrene egg cartons, might have entered the eggs through the feed of the laying hens.

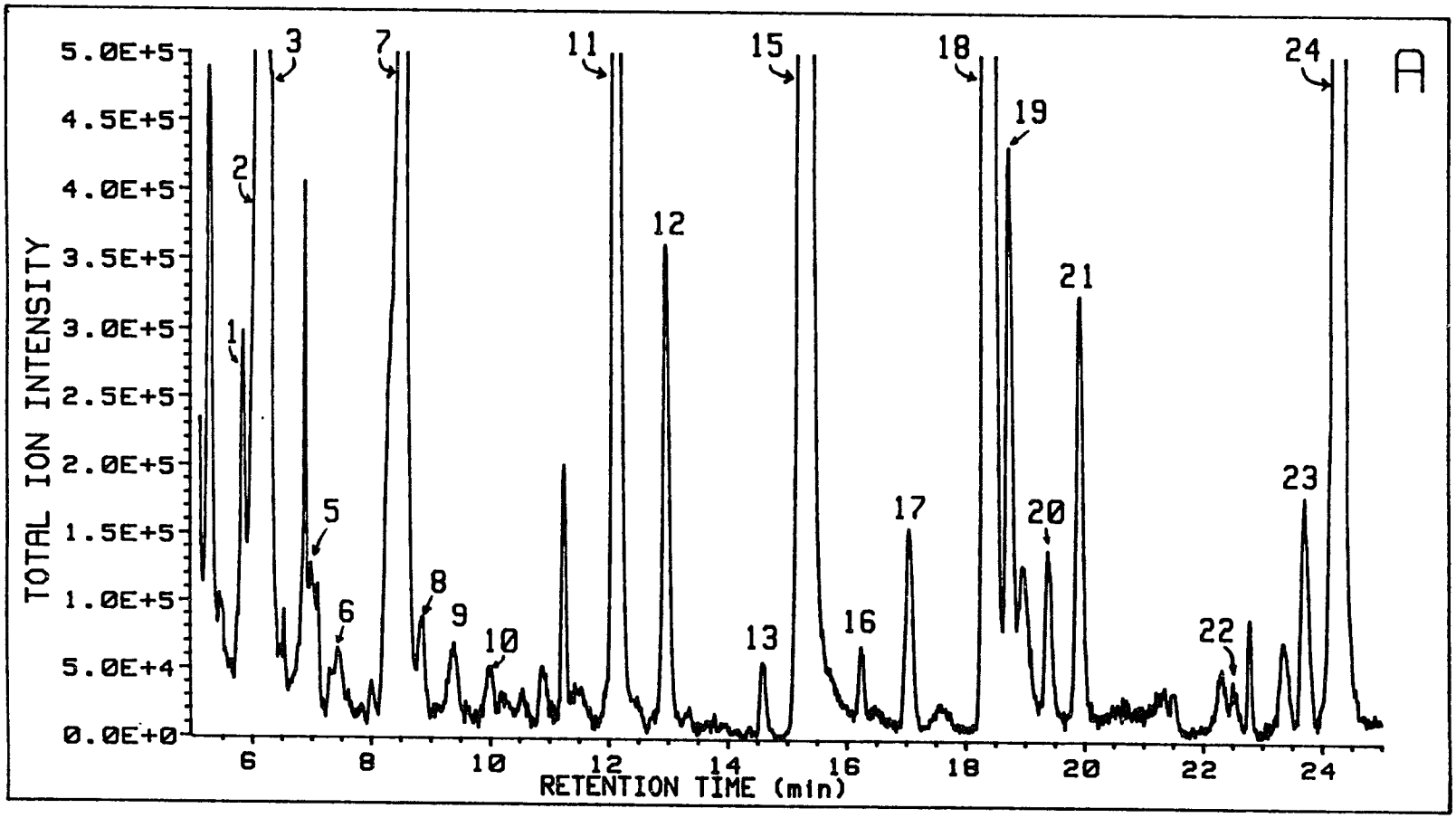
Four n-alkanes including nonane, decane, undecane, and tetradecane were identified in the egg samples. MacLeod and Cave (1975 and 1976) and Umano et al. (1990) also reported the presence of some n-alkanes in their egg samples. Flath et al. (1982), Grosch (1982) and Vejaphan et al. (1988) observed that n-alkanes do not contribute to food flavor.

Two sulfur-containing compounds were detected in the egg samples only. Dimethyl disulfide was present only in batch S eggs at a concentration of 3 ng/g. This compound has been identified in cooked eggs by Macleod and Cave (1975 and 1976) and Umano et al. (1990). MacLeod and Cave (1975) described the odor of dimethyl disulfide as sulfurous, bad eggs. Others have described it as onion- or cabbage-like (Fors, 1983; Vejaphan et al.,1988). Tetrahydrothiophene, which has not been reported in egg samples before, was detected only in batch B eggs. Its relative abundance apparently increased during storage.

Limonene has previously been reported in egg samples by Umano et al. (1990). This compound also was detected in the cooking oil and the polystyrene egg container.

The number of volatile compounds identified in this study was substantially smaller than those reported by previous researchers. However, the DHS approach of sampling may afford more realistic profiles of the volatile compounds present since DHS does not subject the samples to high temperatures for extended time periods. Such treatments tend to generate numerous degradation products that would not occur in eggs cooked in customary ways. Comparison of the volatile composition of egg and packaging material samples suggests that migration of volatile compounds may have occurred from the packaging material into the eggs, and vice versa.

Figure 4 (A-B). Total ion chromatogram of dynamic headspace volatile components of scrambled eggs. Compounds are identified by peak numbers shown in Table 2.


TOTAL ION INTENSITY
5.0E+5
4.5E+5
4.0E+5
3.5E+5
3.0E+5
2.5E+5
2.0E+5
1.5E+5
1.0E+5
5.0E+4
0.0E+0
6
8
10
12
14
16
18
20
22
24
RETENTION TIME (min)
1
2
3
5
6
7
8
9
10
11
12
13
15
16
17
18
19
20
21
22
23
24
A

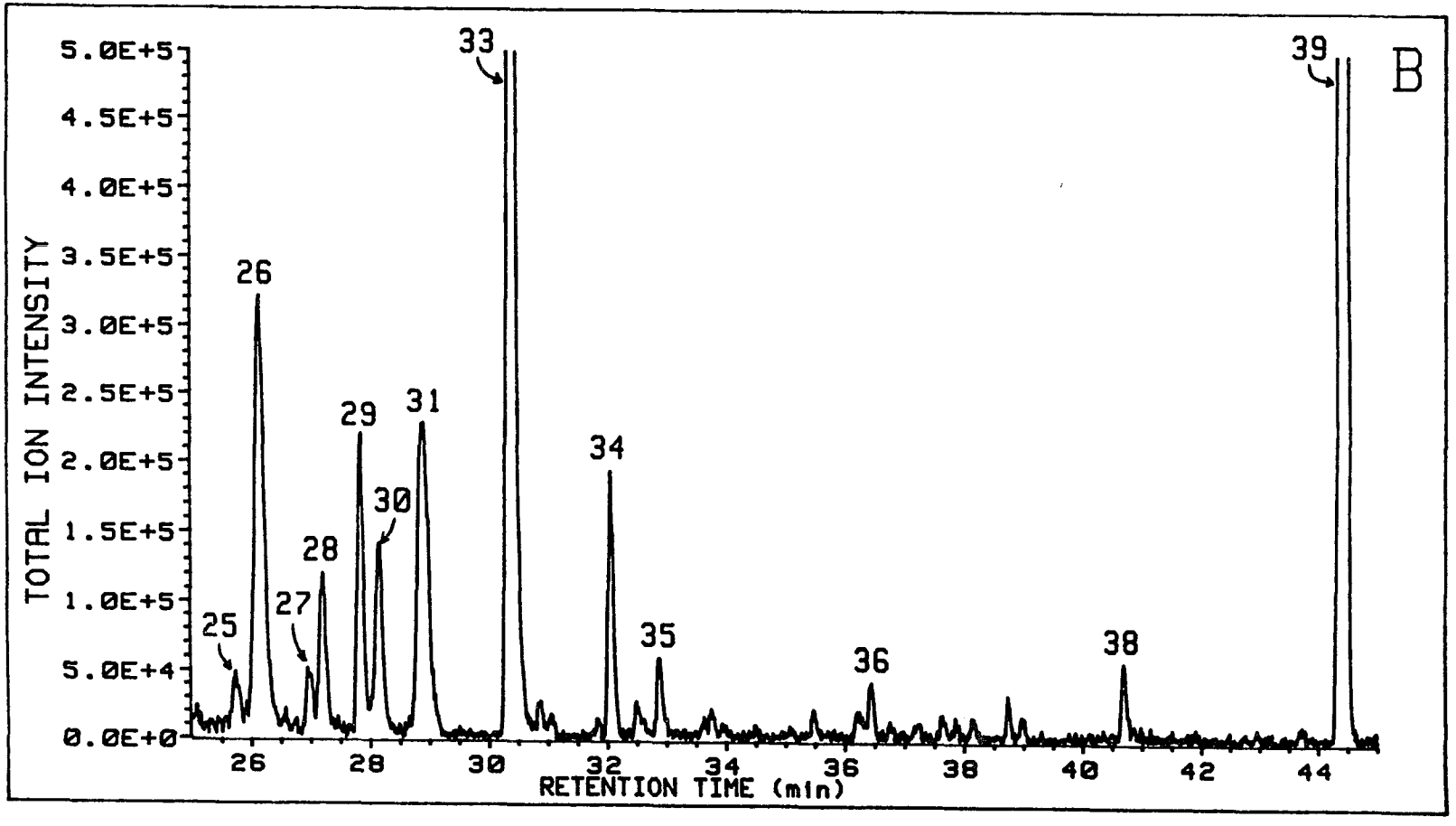

B
TOTAL ION INTENSITY
5.0E+5
4.5E+5
4.0E+5
3.5E+5
3.0E+5
2.5E+5
2.0E+5
1.5E+5
1.0E+5
5.0E+4
0.0E+0
25
26
27
28
29
30
31
33
34
35
36
38
39
26
28
30
32
34
36
38
40
42
44
RETENTION TIME (min)

Table 2. Volatile compounds identified in scrambled eggs.

Peak No.	Compound name by class	RI	EGG SAMPLE BATCH S Area ratio	S Conc ng/g	A Area ratio	A Conc ng/g	B Area ratio	B Conc ng/g
	ALDEHYDES							
2	2-methylbutanal	913	0.13		n.d.		0.08	
3	3-methylbutanal	914	1.86	122	1.92	126	0.32	16
7	pentanal[b]	976	0.21	13	0.40	26	0.43	28
15	hexanal[b]	1088	0.80	39	0.67	32	1.16	59
35	octanal	1295	n.d.		n.d.		0.01	
38	nonanal	1401	0.02		n.d.		0.01	
	KETONES							
10	4-methyl-2-pentanone	1008	0.08	3	0.09	3	0.02	<1
12	3-hexanone	1053	0.06	<1	0.05	<1	0.08	1
	ALCOHOLS							
4	2-propanol	925	n.d.		0.14		0.01	
26	3-hexanol*	1220	n.d.		n.d.		0.08	
30	2-hexanol*	1241	n.d.		n.d.		0.03	
32	1-pentanol	1262	n.d.		0.01		0.01	
	FURANS							
6	2-ethylfuran	951	n.d.		0.03		0.02	
29	2-pentylfuran	1238	0.03	<1	0.06	2	0.06	2
	ESTERS							
8	methyl butyrate	989	n.d.		n.d.		0.02	
13	butyl acetate	1076	n.d.		n.d.		0.01	
	BENZENE DERIVATIVES							
5	benzene[b]	934	0.10		0.70		0.03	
11	toluene	1040	0.59		0.73		0.37	
18	ethylbenzene-d10 (I.S.)	1126	1.00		1.00		1.00	
19	ethylbenzene	1130	0.77	28	0.10	4	0.12	4
20	p-xylene	1138	0.09		0.08		0.04	
21	m-xylene	1144	0.21		0.24		0.09	
22	isopropylbenzene	1178	0.06		n.d.		0.01	
23	o-xylene	1191	0.12		0.09		0.06	
25	propylbenzene	1214	0.02		0.01		0.01	
27	4-ethyltoluene	1228	0.03		0.02		0.01	
28	3-ethyltoluene	1231	0.06		0.05		0.03	
31	1,3,5-trimethylbenzene	1249	0.01		0.01		0.01	
33	styrene	1267	2.74	103	0.03	1	0.38	14
34	1,2,4-trimethylbenzene	1285	0.06		0.03		0.05	
36	1,2,3-trimethylbenzene	1343	0.01		<0.01		0.01	
39	1,4-dichlorobenzene[b]	1451	0.59	18	1.09	33	0.92	28

Table 2. Continued.

Peak No.	Compound name by class	RI	EGG SAMPLE BATCH S Area ratio	S Conc ng/g	A Area ratio	A Conc ng/g	B Area ratio	B Conc ng/g
	ALKANES							
1	nonane	900	0.03		0.01		0.05	
9	decane	1000	0.01		0.02		0.03	
16	undecane[b]	1100	0.02		0.01		0.02	
37	tetradecane	1400	0.01		n.d.		n.d.	
	SULFUR-CONTAINING							
14	dimethyl disulfide	1077	0.07	3	<0.01	<1	n.d.	0
17	tetrahydrothiophene	1110	n.d.		n.d.		0.04	
	TERPENE							
24	limonene	1198	0.28	8	0.04	1	0.65	19

RI = Retention index.
S = Batch S eggs: Supermarket sample packaged in polystyrene.
A = Batch A eggs: fresh farm eggs, not stored.
B = Batch B eggs: fresh farm eggs stored in a polystyrene container for 2 weeks before cooking.
I.S. = Internal standard.
Area ratio = compound peak area/I.S. peak area.
Conc = concentrations of selected compounds.
n.d. = not detected.
* = tentatively identified by MS data only.
[b] = background subtracted. Compounds without superscript [b] were not found in the background (blank runs).

Table 3. Volatile compounds identified in both the scrambled egg and heated corn oil samples.

Compound name	HEATING TIME			
	30 sec		60 sec	
	Mean area ratio	conc ng/g	Mean area ratio	conc ng/g
nonane	0.07		0.16	
2-ethylfuran	0.02		0.03	
pentanal	0.28	1043	0.30	1109
decane	0.03		0.03	
hexanal	1.22	3720	1.01	3039
undecane	0.01		0.02	
heptanal	0.13		0.05	
limonene	0.03	59	<0.01	9
2-pentylfuran	0.06	93	0.02	7
1-pentanol	0.30		0.13	
octanal	0.01		<0.01	
nonanal	0.06		0.01	

Area ratio = compound peak area/I.S. peak area.

Table 4. Volatile compounds identified in polystyrene egg carton.

Compound name	RI	Mean area%
octane	800	0.1
acetone*	819	0.5
butanal*	877	0.2
nonane	900	0.1
3-methylbutanal	912	0.1
2-propanol	925	0.3
dichloromethane	930	0.2
benzene	934	0.8
pentanal	976	0.5
trichloroethene	996	0.2
decane	1000	0.2
chloroform	1024	0.4
toluene	1040	3.6
3-hexanone	1053	0.2
hexanal	1088	0.9
undecane	1100	0.1
ethylbenzene	1130	25.6
p-xylene	1138	0.3
m-xylene	1144	0.8
isopropylbenzene	1178	3.4
o-xylene	1191	0.3
heptanal	1195	0.1
limonene	1198	0.2
propylbenzene	1214	1.1
4-ethyltoluene	1228	0.2
3-ethyltoluene	1231	0.5
1,3,5-trimethylbenzene	1249	<0.1
1-pentanol	1262	0.1
styrene	1267	25.6
2-ethyltoluene	1268	0.1
2-propenylbenzene*	1272	1.2
1,2,4-trimethylbenzene	1285	0.1
octanal	1295	<0.1
2-methyl-1-pentanol	1305	0.1
cyclopropylbenzene*	1338	0.6
1,2,3-trimethylbenzene	1343	<0.1
nonanal	1397	0.2
tetradecane	1400	0.2
1,4-dichlorobenzene	1451	21.6
pentadecane	1500	0.2
benzaldehyde	1530	2.7
phenylethanone*	1664	0.1

* = tentatively identified by MS only.

CHAPTER IV

ANALYSIS OF DIMETHYL SULFIDES AS INDICATORS OF EGG PRODUCT QUALITY

José E. Matiella and Thomas C.-Y. Hsieh

ABSTRACT

A USDA method of dynamic headspace sampling/gas chromatography was evaluated and used to quantify dimethyl sulfides as quality markers in liquid whole egg samples. Percent acceptance (0, 20, 40, 60, 80, or 100%) by a panel of five USDA egg inspectors on an experimental batch of samples generally decreased with increasing dimethyl sulfide (DMS) concentration (0 to 540 ng/g) in the samples. In addition, dynamics of DMS and dimethyl disulfide (DMDS) formation during egg spoilage were investigated. It was found that egg decomposition may follow different paths and that DMS concentration may not be indicative of the extent of spoilage in a sample.

INTRODUCTION

The quality grading of liquid egg products for human consumption in the U. S. is partly based on the subjective sensory evaluation of the odors of the products by licensed U. S. Department of Agriculture (USDA) egg product inspectors (Brown et al., 1986). An objective method to complement and confirm the assessment of egg product quality is necessary when egg producers are in disagreement with the inspectors' judgments. These conflicts often involve considerable losses on the producers' part and litigation against USDA inspectors. Literature on the volatile compounds from decomposing eggs is scarce. Bullard et al. (1978) used a headspace sampling technique to analyze the volatile compounds of fermented eggs. Brown et al. (1986) used gas chromatography (GC) and a flame photometric detector (FPD) to selectively analyze the sulfur-containing compounds, including dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS), that form in eggs as they deteriorate.

The objectives of this investigation were (1) to evaluate the method of analysis described by Brown et al. (1986), (2) to establish a correlation between the concentrations of sulfides in egg samples and their acceptability as determined by a panel of five USDA egg

inspectors, and (3) to study the dynamics of the formation of DMS and DMDS in eggs during spoilage.

MATERIALS AND METHODS

Analysis of USDA expert panel-evaluated egg samples.

Sample preparation. Samples of whole liquid eggs, first evaluated in 1987 by an expert sensory panel of five USDA egg inspectors as described by Morris (1987) were obtained in a frozen (-25°C) state in 1989 from the USDA, Agricultural Research Service, Southern Regional Research Center (New Orleans, LA). Results of the panel's evaluations of the samples were provided by USDA. Each of the egg samples obtained from USDA was originally packed in a covered plastic cup (Dixie/Marathon Products, James River Corp., Norwalk, CT) and kept frozen. To minimize the introduction of sampling variables such as loss of volatile compounds and analytical reproducibility, five 1-g subsamples were taken at once from five locations around the center of each sample cup, tranferred to glass vials (17 mm o.d. x 60 mm height) fitted with Teflon cap liners, and stored at -25°C until analysis. Immediately before GC analysis, the subsamples were prepared for dynamic headspace sampling (DHS) as follows:

1. A subsample was taken from the freezer and thawed by warming the vial between the hands.

2. Using a Pasteur pipette fitted with a rubber bulb, a 0.1g egg sample was weighed (to the nearest 0.0001g) into a dynamic headspace sample tube. The desired sample weight was obtained by transferring 4-5 drops of the egg liquid.

3. Sample weight was recorded, and the mouth of the sample tube was covered with Parafilm^{TM} to minimize losses of volatiles until the sample tube was attached to the dynamic headspace sampling (DHS) system (maximum of 5 minutes after weighing). Three subsamples from each USDA sample cup were analyzed.

Dynamic Headspace Sampling/Gas Chromatography/Flame Photometric Detection (DHS/GC/FPD).

The sample tube was attached to a DHS system consisting of a Tekmar DHS sample fitting/sparging needle set (part No. 14-3964-100, Tekmar Co., Cincinnati, OH) connected to the GC injector port loop through a 6-port, 2-position Valco valve (valve product No. C6T, Valco Instruments Co., Inc., Houston, TX). Helium (99.999%) was used as the purging and cleaning gas for DHS, and also as the GC carrier gas. Egg sample volatile compounds were purged at room temperature using a purge gas flow rate of 30 mL/min

for 45 min. The sample fitting, transfer lines, and the 6-port valve were kept at 125°C during purging and analysis. The GC injector port was kept at 200°C and the detector at 250°C. The volatile compounds purged from the egg samples were trapped by the GC column packing material. A Hewlett-Packard HP5890 GC equipped with a stainless steel column (2.159 mm i.d. x 2.44 m length), packed with 7% poly-m-phenoxylene (poly-MPE) on Tenax-GC (80/100 mesh), was utilized. A flame photometric detector (FPD) (Hewlett-Packard Co., Palo Alto, CA) was used for selective detection of sulfur compounds. The GC oven temperature was programmed to remain at 30°C for 45 min (during sample purge), then the temperature was increased to 60°C at a rate of 30°C/min, and to 255°C at a slower rate of 15°C/min. The final temperature was maintained for an additional 10 minutes (total run time = 69 min). DMS and DMDS were identified by matching retention times with those of authentic DMS and DMDS standards. This procedure was similar to the one developed by Brown et al. (1986), except in the following areas: smaller sample amount; no water or antifoam addition; no sparging but purging; greater purge flow rate; and longer sampling time.

Dynamics of DMS and DMDS in eggs during spoilage.

Two egg spoilage studies were conducted to investigate the formation of DMS and DMDS; and to test the sensitivity and reproducibility of the above described DHS/GC/FPD method. In the first spoilage study, a batch of fresh supermarket eggs was shelled, blended, and allowed to spoil for six days at room temperature (20 to 25^{o}C), with constant agitation. Sampling aliquots (1g each) were taken every 24 hrs and stored at -85^{o}C. In the second egg spoilage study, fresh supermarket eggs were allowed to spoil for 12 days under the same conditions, but sampling aliquots were taken every 12 hours. Samples were analyzed in quadruplicate for sulfides by the DHS/GC/FPD method described above.

Determination of calibration curves for DMS and DMDS.

To account for egg matrix effects on the detection of DMS and DMDS, standard solutions containing various known levels of authentic DMS and DMDS were added to portions of whole homogenized fresh farm eggs (laid on the same day) obtained from the LSU Poultry Farm. Each spiked egg portion was divided into 1-g aliquots placed in vials, and frozen in the same manner as the USDA samples. Four aliquots of each standard concentration level were analyzed. The concentration range of the

standards was adjusted such that the peak areas of the DMS and DMDS from the USDA inspected samples could be covered by the standard curves. Regression equations that best described the relationship between concentration and peak area were calculated. The equations were tested by substituting peak area/g of sample values in each equation to back-calculate the concentrations of sulfides in the standards.

RESULTS AND DISCUSSION

Analysis of USDA egg samples

Calibration curves. The calibration curves determined for DMS and DMDS are shown in Figure 5. Both were best described by quadratic equations. Narziss et al. (1983) reported that the FPD response to increasing concentrations of sulfur compounds is an exponential function of the concentration, and that log-log plots of detector response vs DMDS concentration gave straight lines. Brown et al. (1986) also have reported that the log-log curves of peak area vs. amount of DMS or DMDS were straight lines with slopes of 1.74 and 1.81, respectively. In this study, however, quadratic equations were better calibration curves for both DMS and DMDS.

DMS and DMDS in the USDA egg samples. A typical FPD chromatogram of sulfur-containing compounds in spoiled egg samples is shown in Figure 6. Mean DMS and DMDS concentrations (ng/g) of the individual USDA egg samples are presented in Table 5, sorted by increasing DMS concentration. Large variation coefficients (CV) of the means of repeat determinations from the same sample were observed in most cases (Table 5), possibly due to loss of volatile DMS and DMDS through leakage between the plastic lid and cup during the long storage period.

Correlation of DMS and DMDS concentrations with acceptance by USDA inspectors.

Results show no apparent correlation between DMS and DMDS concentrations, or between DMDS concentration and percent acceptance by the sensory panel (Table 5). Therefore, DMDS appeared to be unreliable as an egg quality marker. Table 6 shows the total number of samples that were accepted by 100, 80, 60, 40, 20, or 0 percent of the expert sensory panel, within four different DMS concentration ranges (0-100, 101-200, 201-300, and 301-550 ng/g). For example, out of 10 egg samples containing DMS within 301 to 550 ng/g (the highest concentration range), there was one sample that was accepted by 80% (4 out of 5) of the panel members

(Table 6). This suggests that, in some cases, egg samples containing high concentrations of DMS may still be judged acceptable by most inspectors. Brown et al. (1986) stated that DMS in egg products judged unacceptable may or may not have contributed to the perceived odor of spoiled eggs. However, percent acceptance appeared to decrease, in general, with increased DMS concentration.

Egg spoilage study I

Figure 7 shows changes in mean peak area for DMS and DMDS as measured by the USDA method during the first egg spoilage study. CV's for these means varied from 5 to 133% (average of 32%, n = 7) for DMS and 7 to 14% (average of 11%, n = 5) for DMDS. Highest CV's were observed in samples containing only trace levels (peak area < 10,000 counts) of these compounds. In this experiment, DMS was detected before DMDS during the course of spoilage. DMDS increased as DMS decreased over time (Figure 7). Large changes in peak areas between sampling points suggested that more frequent sampling may be necessary to better monitor these changes. It also was apparent that the study was not long enough to show changes in DMDS beyond 144 hrs. For these reasons, sampling frequency and experimental period were doubled for the second spoilage study.

Egg spoilage study II

Changes in mean DMS and DMDS peak areas during the second egg spoilage study are presented in Figure 8. CV values ranged from 5 to 65% (average of 19%, n = 25) for DMS and 6 to 128% (average of 31%, n = 23) for DMDS, depending on the abundance of each compound.

A comparison of results in Figures 7 and 8 shows that the sulfides did not behave the same way in both egg spoilage studies. In the first study, DMS was detected before DMDS and its peak area was highest at 48 hrs. In the second spoilage study, DMS appeared after DMDS, and its highest peak area was detected at 96 hrs.

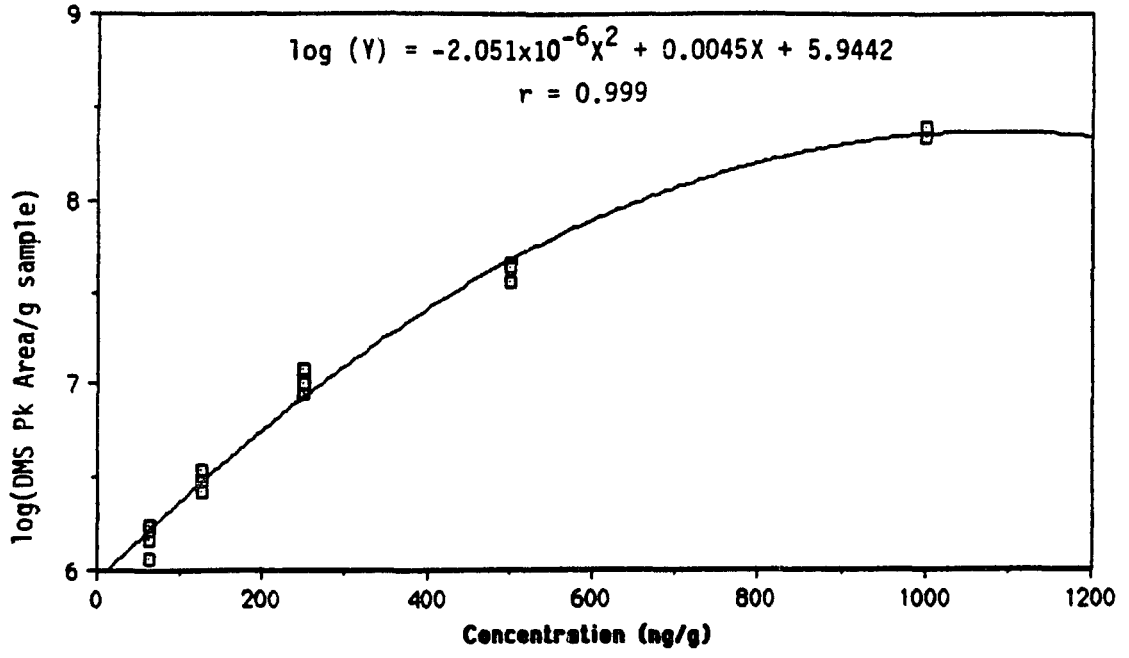
CONCLUSIONS

Results from the analyses of USDA liquid egg samples indicated that, as suggested by Brown et al. (1986), DMS may serve as a chemical indicator for the objective evaluation of the quality of egg products. However, egg spoilage may follow different paths, and DMS may not appear early enough nor increase in concentration throughout spoilage to enable this indicator to serve as a wide range quality marker in a consistent manner. More research with more careful handling and shorter storage of sensory-evaluated egg

samples is recommended to reach more definite conclusions on the reproducibility of the USDA analytical method and the usefulness of DMS as an egg quality marker.

Figure 5. FPD calibration curves determined for calculation of DMS and DMDS in liquid egg products.

DMS Calibration Curve



DMDS Calibration Curve

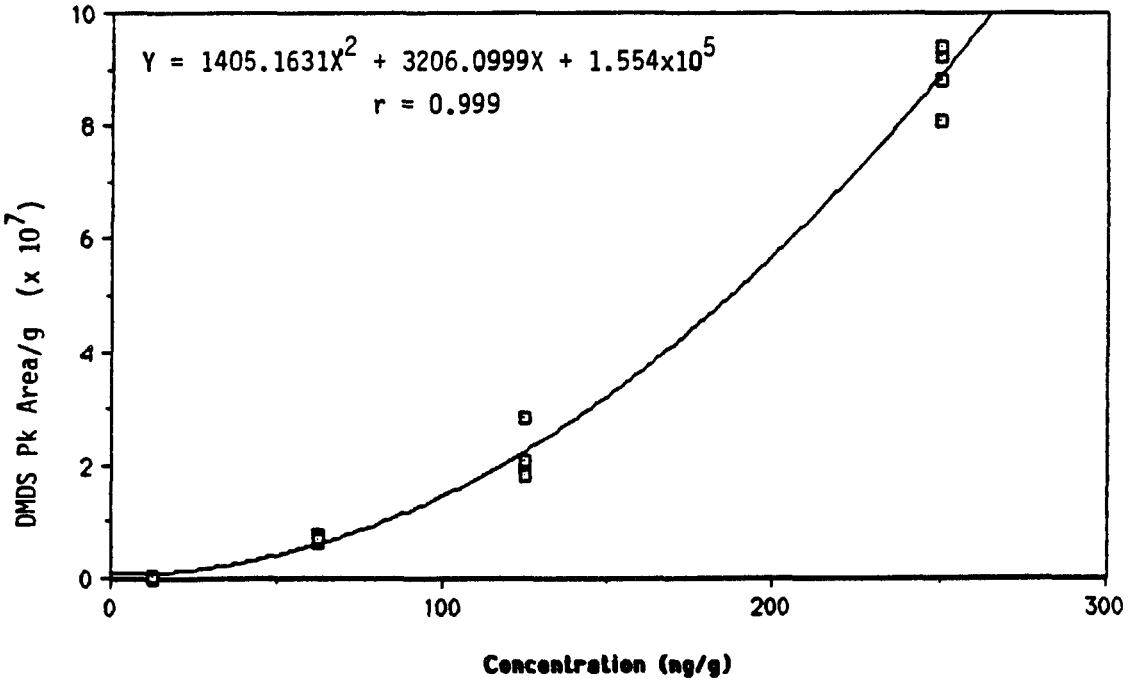


Figure 6. Typical FPD chromatogram of sulfur-containing volatile compounds in spoiled eggs.

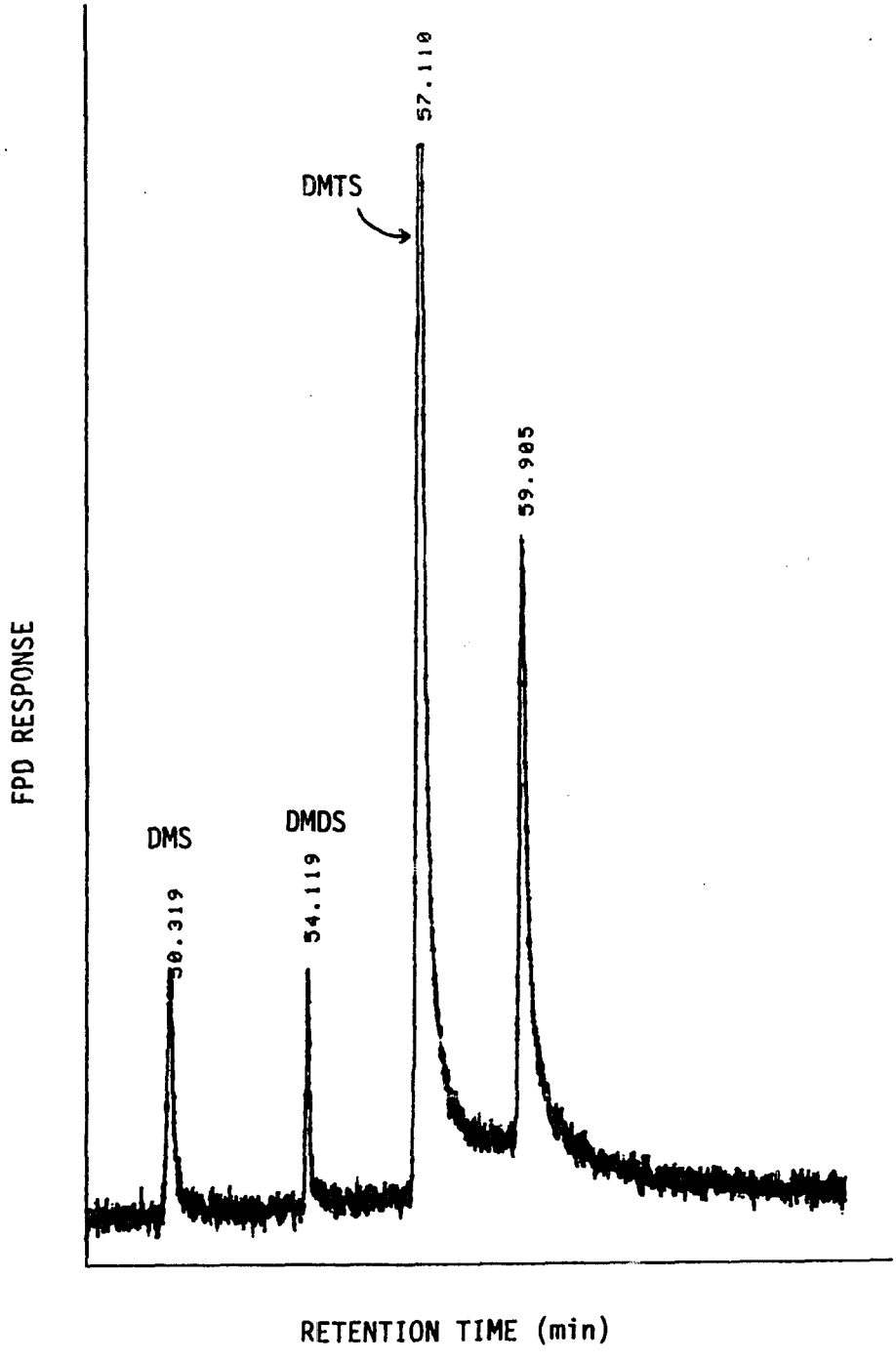

FPD RESPONSE
DMS
50.319
DMDS
54.119
DMTS
57.110
59.905
RETENTION TIME (min)

Figure 7. Changes in DMS and DMDS absolute peak area during the first egg spoilage study.

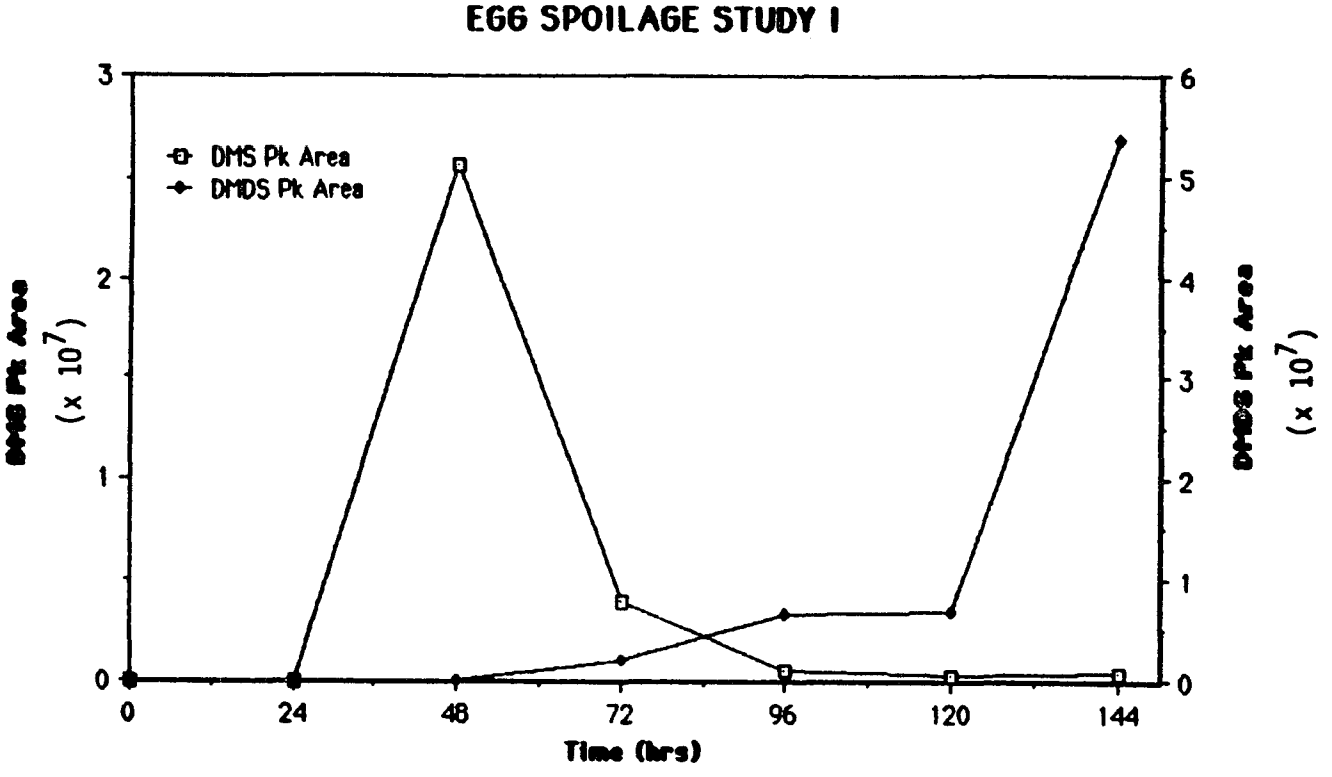

EGG SPOILAGE STUDY I
DMS Pk Area
DMDS Pk Area
DMS Pk Area (x 10^7)
DMDS Pk Area (x 10^7)
Time (hrs)
0
24
48
72
96
120
144
0
1
2
3
4
5
6

Figure 8. Changes in DMS and DMDS absolute peak area during the second egg spoilage study.

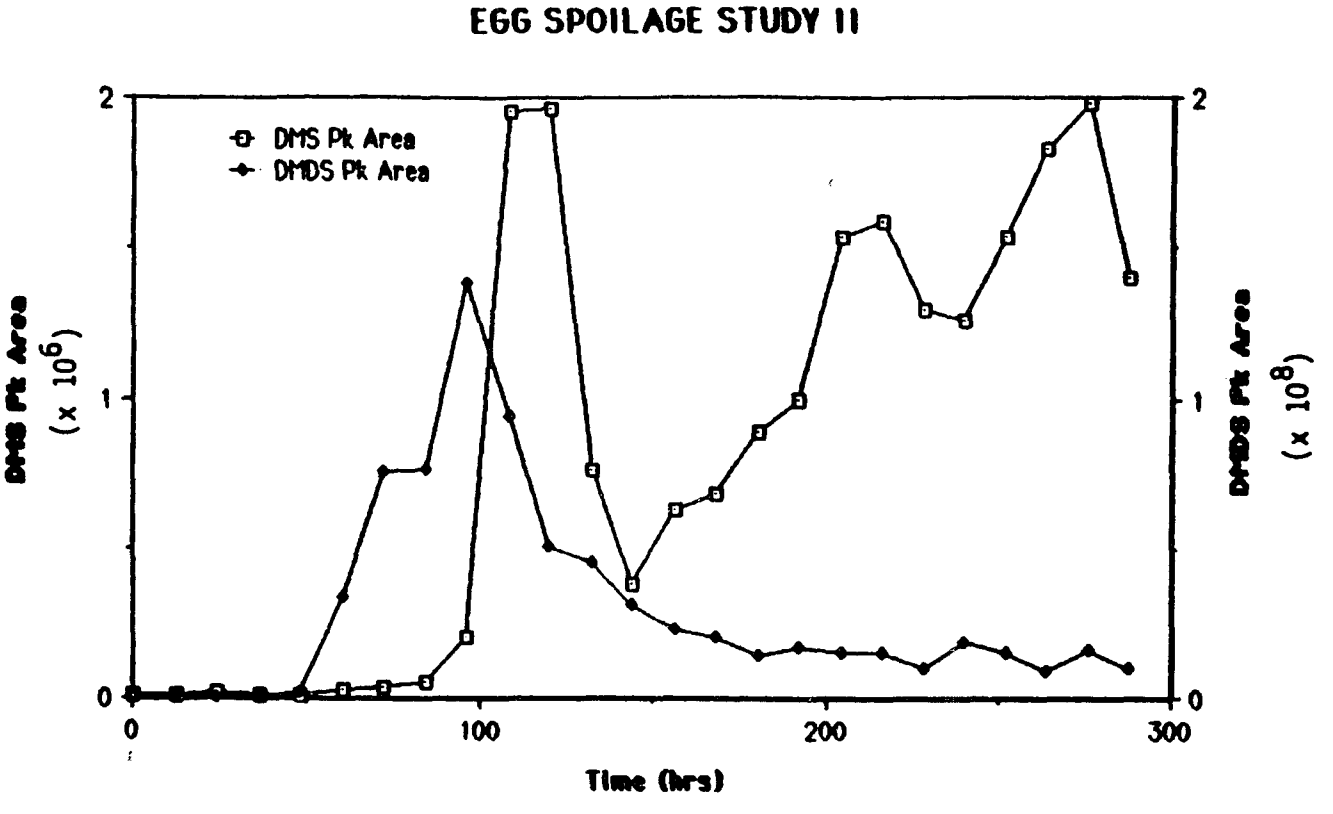

EGG SPOILAGE STUDY II
DMS Pk Area
DMDS Pk Area
DMS Pk Area (x 10^6)
DMDS Pk Area (x 10^8)
Time (hrs)
0
100
200
300
1
2

Table 5. Analyses of whole egg samples evaluated by the USDA expert sensory panel.

Lot No.	Sample No.	Mean DMS Conc (ng/g)	DMS CV %	Mean DMDS Conc (ng/g)	DMDS CV %	%ACCEPT BY PANEL
6	784	n.d.	-	n.d.	-	100
1	221B	n.d.	-	n.d.	-	100
1	468	n.d.	-	n.d.	-	100
5	624	n.d.	-	n.d.	-	100
11	744	n.d.	-	n.d.	-	100
3	993	n.d.	-	n.d.	-	100
4	062	n.d.	-	n.d.	-	100
6	376	n.d.	-	1	173	100
6	725	n.d.	-	n.d.	-	100
2	943	n.d.	-	n.d.	-	100
1	659	n.d.	-	n.d.	-	100
3	137	n.d.	-	n.d.	-	100
1	768	n.d.	-	n.d.	-	100
9	733	n.d.	-	n.d.	-	100
4	478	n.d.	-	n.d.	-	100
2	272	n.d.	-	n.d.	-	100
11	754	n.d.	-	n.d.	-	100
1	221	n.d.	-	n.d.	-	100
5	521	n.d.	-	n.d.	-	100
8	269	n.d.	-	3	174	100
9	608	n.d.	-	n.d.	-	100
2	648	n.d.	-	n.d.	-	100
5	328	n.d.	-	n.d.	-	100
12	390	n.d.	-	n.d.	-	80
2	950	n.d.	-	n.d.	-	80
5	280	n.d.	-	n.d.	-	80
2	610	n.d.	-	n.d.	-	80
5	259	n.d.	-	n.d.	-	80
3	539	n.d.	-	n.d.	-	80
7	639	n.d.	-	5	173	60
3	773	n.d.	-	2	173	40
3	751	n.d.	-	8	32	0
12	710	31	98	8	120	100
6	120	51	57	4	134	40
9	305	89	96	n.d.	-	20
9	040	110	173	5	173	0
8	199	136	49	8	18	20
9	569	145	20	n.d.	-	0
4	416	160	17	18	23	0
6	449	194	20	1	174	0
8	812	219	36	1	173	60
2	248	253	27	n.d.	-	0
5	711	259	36	2	91	0
1	952	277	42	5	69	0

Table 5. Continued.

Lot No.	Sample No.	Mean DMS Conc (ng/g)	DMS CV %	Mean DMDS Conc (ng/g)	DMDS CV %	%ACCEPT BY PANEL
9	732	284	14	n.d.	-	80
4	351	291	24	n.d.	-	80
6	474	297	30	n.d.	-	60
12	637	300	40	2	174	0
4	232	314	28	1	174	20
8	210	331	26	4	173	0
11	364	336	47	15	73	0
4	627	354	6	4	97	80
7	961	363	2	n.d.	-	0
12	350B	416	8	n.d.	-	0
5	316	422	5	2	173	40
5	711B	444	13	n.d.	-	0
12	350	505	23	n.d.	-	0
11	225	540	18	n.d.	-	0

Conc = concentration.
n.d. = not detected.

Table 6. Frequency distribution of sensory panel acceptance of USDA egg samples by four DMS concentration ranges.

DMS Conc Range	Num. of samples in range	NUMBER OF SAMPLES BY PERCENT ACCEPTANCE BY EXPERT PANEL					
		100%	80%	60%	40%	20%	0%
0-100	35	24	6	1	2	1	1
101-200	5	0	0	0	0	1	4
201-300	8	0	2	2	0	0	4
301-550	10	0	1	0	1	1	7

Conc = Concentration (ng/g).

CHAPTER V

OVERALL SIGNIFICANCE AND CONCLUSIONS.

Existing dynamic headspace sampling equipment and methodology were effectively modified and successfully applied to the analysis of volatile compounds from two complex high-moisture/protein-rich food matrices (crabmeat and eggs).

A simplified purge-and-trap system (Figure 1) was designed, built, and successfully utilized in the research projects presented in chapters II and III. This system has the following advantages:

Low cost. This device offered an economic alternative to commercial automated DHS instruments; and it afforded effective sampling of volatile compounds.

Simplicity. The system does not include complex electronic devices that may be expensive and time-consuming to repair in case of malfunctions. Minimum plumbing eliminated the need for long heated transfer lines and multiple port valves. This simplicity in design also facilitated disassembly and reassembly of the system for cleaning and repair when necessary.

It was found that highly perishable samples (e.g. crabmeat) spoiled during the 6-hr sampling period when

they were loaded to the 10-position DHS system all at once to purge in the automatic mode. Soilage generated a number of volatile compounds that were not in the "fresh" sample. The following improvements were made to the DHS methods used by previous researchers in our department:

1. The method was improved so that aliquots of sample homogenate were thawed immediately before purging and purged one at a time to prevent spoilage.

2. Liquid nitrogen was used instead of dry ice/ethanol to cryogenically focus the volatile compounds at the beginning of the GC column. This decreased the cryofocusing temperature from -84^{o}C to -200^{o}C, minimizing volatile compound breakthrough. Although the effect of lowering the cryofocusing temperature on minimizing trap breakthrough was not quantified, the use of liquid nitrogen to replace the dry ice/ethanol bath eliminated the possibility of dry ice pieces scratching the surface of the fused silica column. The risk of column breakage also was diminished.

Chapter II presents the first article (in press) reporting specific chemical data on volatile compounds from blue crab meat. Fifty-three volatile compounds were identified in crabmeat samples. Levels of volatile compounds in both samples were compared and it

was found that the boiled crabmeat contained higher levels of most of the compounds identified. This suggested that further processing (such as pasteurization) and/or storage may have decreased the abundances of volatile flavor compounds, which may negatively affect the flavor quality of the crabmeat.

Chapter III presents the first analytical research paper on volatile compounds from scrambled eggs. This paper also reports the phenomenon of volatile compound migration through the egg shell, from an egg container, and vice versa. Thirty-eight volatile compounds were identified in scrambled eggs. Selected compounds were quantified. Aldehydes were found to be the most abundant volatile compounds in the scrambled egg samples. The concentration of volatile styrene monomer increased in scrambled egg samples during a two-week storage of fresh farm shell eggs in a polystyrene egg carton. Scrambled eggs prepared from a batch of supermarket shell eggs stored in a polystyrene egg carton contained seven times more ethylbenzene and styrene than those prepared from a batch of fresh farm eggs stored in a polystyrene container at $4^{o}C$ for two weeks. High levels of these benzene derivatives in foods are undesirable from both the flavor quality and health points of view. Prolonged storage at elevated temperatures may increase the rate of volatile compound

migration. Comparison of the volatile composition of scrambled egg and packaging material samples suggested that migration of volatile compounds may have occurred from the packaging material into the eggs, and vice versa. The number of volatile compounds identified in scrambled eggs was substantially smaller than those reported in egg samples by previous researchers. However, the DHS approach of sampling utilized in this study may afford more realistic profiles of the volatile compounds present in the samples since DHS does not subject the samples to high temperatures for extended time periods during the analytical procedure. Such treatments reported in the literature before this study tend to generate numerous degradation products that would not occur in eggs cooked in customary ways. Therefore, such products must be considered analytical artifacts.

Chapter IV reports the evaluation and modification of a USDA-ARS-SRRC-developed DHS/GC method for the analysis of volatile sulfur-containing compounds (dimethyl sulfide and dimethyl disulfide) as quality indicators for objective assessment of the quality of liquid egg products. Objective analytical methods of this type are very much needed to complement and confirm results from the current subjective odor-based quality grading of liquid egg products for human

consumption. This subjective inspection is performed by licensed USDA egg product inspectors. However, the current grading method often produces inspection results far from convincing to egg producers and the conflicts often lead to court litigations.

The USDA DHS/GC method was modified as follows: (1) The sample amount used in each analysis run was decreased from 1.0 to 0.1 g to reduce the thickness of the sample matrix and allow more consistent purging efficiency. The purge gas flow rate was increased from 14 to 30 mL/min and purging time was increased from 30 to 45 min, which allowed more efficient sweeping of volatile sulfur compounds from the egg matrix. (2) Purging of headspace volatile compounds was used instead of sparging (bubbling purge gas directly into the egg sample), reducing the possibility of contamination of sampling equipment and eliminating the need for lengthy and tedious equipment cleaning after each analysis. This also eliminated the need for water and antifoam addition which would make the matrix more complex. (3) Calibration curve standards were prepared by spiking the liquid whole egg matrix with various known amounts of standard compounds in order to account for matrix effects as closely as possible. The

original USDA method did not use the egg matrix in preparing the standard calibration curve.

The modified USDA method produced adequate measurements of dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) in the spoiling liquid egg samples. Furthermore, analyses of the USDA egg samples showed a trend indicating that sample acceptability (as judged by a panel of five USDA egg inspectors) generally decreased with increasing DMS concentration in the samples. However, egg spoilage studies described in chapter IV revealed that DMS and DMDS do not always behave the same way in spoiling liquid eggs. This suggested that egg spoilage may follow different paths, depending on the type of microflora responsible for spoilage. Because of this, DMS may not appear early enough nor increase in concentration consistently throughout the egg sampling period for inspection to enable this indicator to serve as a wide range quality marker in a dependable manner. To investigate and achieve full understanding of liquid egg spoilage microbiology and its effects on the production of DMS and DMDS would require microbiological and chemical research using pure and mixed cultures of micro-organisms commonly found in egg samples. This type of detailed microbiological study is beyond the scope of this research project.

In this age of processed and packaged foods, specific chemical information on the volatile flavor components of foods is of extreme value. A food that does not have good flavor will not sell, regardless of how nutritious, sanitary, well packaged, or well presented the product may be. Also, the composition of volatile compounds in a food system often reveals the nature and history of the food's ingredients. Many food companies have understood this and are now paying very much attention to the analytical study of the flavor chemistry of their products. Information of this type can be utilized to complement sensory panel findings (1) to establish objective in-house flavor quality standards for product maintenance; (2) to do trouble-shooting when flavor problems occur either with existing or developing products; (3) to study product flavor shelf life; and (4) to monitor changes in competitive products.

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APPENDICES

APPENDIX A

List of experimental GC/MS data files used in chapter II.

TYPE OF CRABMEAT	
BOILED	PASTEURIZED
JMCRAB4.D	JMCRAB8.D
JMCRAB5.D	JMCRAB9.D
JMCRAB6.D	JMCRAB10.D

APPENDIX B

List of experimental GC/MS data files used in chapter III.

EGG SAMPLE BATCH		
S	A	B
JMEGG1.D	JMEGG5.D	JMEGG15.D
JMEGG2.D	JMEGG6.D	JMEGG16.D
JMEGG4.D	JMEGG8.D	JMEGG17.D

APPENDIX C

Calibration curve data used in USDA egg sample analyses.

GC RUN FILE NAME	STD CONC. ng/g (ppb)	DMS AREA/ g SAMPLE	DMDS AREA/ g SAMPLE	SAMPLE WEIGHT (g)	CALC* DMS (ng/g)	CALC* DMDS (ng/g)
CALIB1	0.00	0	0	0.1171	-	-
CALIB2	0.00	0	0	0.1186	-	-
CALIB3	0.00	0	0	0.1143	-	-
CALIB4	0.00	0	0	0.1200	-	-
CALIB5	6.25	0	0	0.1200	-	-
CALIB6	6.25	0	0	0.1159	-	-
CALIB7	6.25	0	0	0.1206	-	-
CALIB8	6.25	0	0	0.1245	-	-
CALIB9	12.50	0	156843	0.1156	-	0.38
CALIB10	12.50	0	379290	0.1197	-	11.53
CALIB11	12.50	0	296419	0.1131	-	8.94
CALIB12	12.50	0	305783	0.1233	-	9.27
CALIB14	62.50	1158599	7773029	0.1228	26.94	72.50
CALIB15	62.50	1460105	6361841	0.1244	50.07	65.33
CALIB16	62.50	1676518	7574966	0.1192	64.14	71.53
CALIB21	62.50	1744199	6790240	0.1124	68.21	67.58
CALIB17	125.00	3471233	28237718	0.1249	141.65	140.23
CALIB18	125.00	2942657	19048729	0.1227	123.52	114.82
CALIB19	125.00	2953365	20712666	0.1174	123.91	119.82
CALIB20	125.00	2660585	18395844	0.1179	112.62	112.80
CALIB22	250.00	10397510	88159903	0.1237	272.14	249.12
CALIB23	250.00	8963247	81116877	0.1044	253.30	238.90
CALIB24	250.00	10142033	92451001	0.1269	268.95	255.15
CALIB25	250.00	11922144	93985336	0.1222	289.89	257.27
CALIB26	500.00	41553491	505997781	0.1226	474.87	598.85
CALIB27	500.00	35086667	477765879	0.1143	446.72	581.87
CALIB28	500.00	44742278	444433297	0.1295	487.58	561.16
CALIB29	500.00	42038154	409768007	0.1219	476.84	538.77
CALIB30	1000.00	222207046	1546216301	0.1141	917.98	1047.80
CALIB31	1000.00	236364582	1449854102	0.1231	959.26	1014.58
CALIB32	1000.00	219130210	1333022940	0.1238	909.92	972.80
CALIB34	1000.00	250767264	1462178531	0.1239	1016.69	1018.89

* Concentration calculated using pertinent regression equation of concentration vs. peak area/g sample.

APPENDIX D

Permission from the Institute of Food Technologists to use the article "Analysis of Crabmeat Volatile Compounds" as part of this dissertation.

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John B. Klis, Director of Publications

March 1, 1990

Mr. Jose E. Matiella
1607 Hunnington Place, #1
Louisville, KY 40220

Dear Mr. Matiella:

Permission is hereby granted to you to use the material requested in your letter of February 12, 1990, as a portion of your Ph.D. dissertation.

Said material is contained in your article, "Analysis of Crabmeat Volatile Compounds" (manuscript No. JFS-770-89-REV-2), recently accepted for publication in the Journal of Food Science.

Very sincerely yours,

John B. Klis
Director of Publications

JBK:eb

FOOD TECHNOLOGY

JOURNAL OF FOOD SCIENCE

VITA

José Ernesto Matiella, son of José Manuel Matiella and Rosa Elvia Dávila, was born on May 10, 1959 in Magdalena, Sonora, Mexico. He completed his secondary education at Preparatoria La Salle, in Mexico City, in 1978.

He entered the Monterrey campus of the Instituto Tecnológico y de Estudios Superiores de Monterrey in January 1979, and transferred to the Guaymas campus in January 1980, where he received the degree of Ingeniero Bioquímico (B.S.), in December of 1982.

He attended graduate school at Auburn University, Auburn, Alabama, where he worked as a research assistant in the Department of Fisheries and Allied Aquacultures. He conducted research under the supervision of Dr. David R. Bayne. His thesis title was "Mixing of Channel Catfish-Hybrid Carp Ponds with Axial Flow Pumps". He received the Master of Science degree from Auburn University in August of 1985.

He worked as a Research Technician at the Texas A&M Shrimp Mariculture Project, Texas Agricultural Experiment Station, from June 1985 to January 1987.

He entered the Ph.D. program in the Department of Food Science at Louisiana State University in January, 1987. He conducted several analytical flavor research projects under the direction of Dr. Thomas C.-Y. Hsieh.

He is a member of the Institute of Food Technologists (IFT) (1987-present) and of the American Chemical Society (1987-present). He was awarded the Mid-South Section IFT Scholarship in 1988, and the national Institute of Food Technologists Fellowship in 1989.

He is currently a candidate for the degree of Doctor of Philosophy in Food Science.

Publications and presentations:

Matiella, J.E. and Hsieh, T.C.-Y. 1990. Analysis of volatile compounds in scrambled eggs. J. Food Sci. Submitted for publication.

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Hsieh, T.C.-Y., Vejaphan, W., Williams, S.S., and **Matiella, J.E.** 1989. Volatile flavor components in

thermally processed Louisiana red swamp crayfish and blue crab. In "Thermal Generation of Aromas" Parliment, T.H., McGorrin, R.J., and Ho, C.-T. (Eds.). ACS Symposium Series No. 409. American Chemical Society, Washington, DC. pp. 386-395.

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Food Science

Title of Dissertation: Sampling and Analysis of Dynamic Headspace Volatile Compounds from Selected Protein-Rich Food Matrices

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

March 23, 1990.